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## Enhancing Herbicide Efficacy on Reed Canary Grass (*Phalaris arundinacea*) by Testing a Plant Growth Hormone, Application Times, and Herbicide Type

Denise Lynn Fong  
*Wright State University*

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**ENHANCING HERBICIDE EFFICACY ON REED CANARY GRASS  
(*Phalaris arundinacea*) BY TESTING A PLANT GROWTH HORMONE,  
APPLICATION TIMES, AND HERBICIDE TYPE**

**A thesis submitted in partial fulfillment of the requirements for the degree  
of Master of Science**

**By**

**Denise Fong  
B.S., The Ohio State University, 2008**

**2013  
Wright State University**

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**DENISE FONG**

**2013**

WRIGHT STATE UNIVERSITY

GRADUATE SCHOOL

March 8, 2013

I HEREBY RECOMMEND THAT THE THESIS PREPARED  
UNDER MY SUPERVISION BY Denise Lynn Fong  
ENTITLED Enhancing herbicide efficacy on reed canary  
grass (*Phalaris arundinacea*) by testing a plant growth  
hormone, application times, and herbicide type BE  
ACCEPTED IN PARTIAL FULFILLMENT OF THE  
REQUIREMENTS FOR THE DEGREE OF Master of  
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## **Abstract**

Fong, Denise Lynn. M.S. Department of Biological Sciences, Wright State University, 2013. Enhancing herbicide efficacy on reed canary grass (*Phalaris arundinacea*) by testing a plant growth hormone, application times, and herbicide type.

*Phalaris arundinacea*, also known as reed canary grass (RCG), is a non-native invasive grass that thrives in floodplains. RCG plants displace native flora which reduces diversity in otherwise species rich wetlands. This is a widespread problem throughout many parts of the USA. RCG can grow by its seeds or its rhizomes. Its seeds are capable of surviving long periods in soil. RCG can potentially be controlled by multi-year treatments in early spring and early fall with broad spectrum or grass specific herbicides. The goal of this study was to optimize methods to control RCG, in order to increase species diversity.

One objective was to examine effects of broad spectrum herbicide AquaNeat® (glyphosate), and grass specific Fusilade II® (fluazifop-p-butyl) applied in either spring or fall, or both spring and fall at two field sites in order to ascertain which combination(s) of treatment can potentially control RCG. Both field sites were in zones affected by floods and contained at least 95% cover of RCG. A second objective asked whether pretreatment with a plant growth hormone called X-Cyte™ (kinetin), shown to release buds from dormancy to enhance impact of

herbicides, would enhance any of the affects revealed by the first seasonal and herbicide based experiments. Based on biomass measurements, single herbicide application in either spring or fall was less effective than treatment in the spring followed by treatment in the fall. Kinetin in the field appeared to have no significant effect on the efficacy of either herbicide type, but this result may be due to the timing of application. Kinetin applied at a lower height in the greenhouse successfully released above ground buds from dormancy and appeared to produce a full kill. However, further observation revealed that rhizomes were not killed. Thus, field results combined with greenhouse results suggest kinetin is of little value in augmenting kill of RCG.

Overall, glyphosate was more successful than fluazifop-p-butyl in reducing RCG biomass and percent cover. This was especially noticeable on a flat site with an initial monoculture of RCG. Treatment of glyphosate in spring and fall did not completely harm desirable plants; a stand of greenheaded coneflowers grew in a treated area where RCG was once a monoculture, probably from below ground dormant material. Spring treatment killed RCG top growth, but it was unclear whether underground rhizomes were killed. RCG sprayed just prior to flowering in summer failed to produce seed. In greenhouse experiments (likely simulating spring conditions), fluazifop-p-butyl treated plants suffered top kill, but all apparently dead rhizomes held at less than -0°C (simulating vernalization) grew new shoots after return to 23°C. This shows that early successful control may be short lived, which would require repeated treatment in successive years.

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Dedication:

To the master key I  
borrowed designed to  
open doors to success.

#ItsMineNow

## **Introduction**

Biological invasions have been a growing problem in the US because invasions are the main cause of diversity loss (Vitousek *et al.*, 1996), and billions of dollars are spent annually for control of invasive species (Pimental *et al.*, 2001). Many parts of the Midwestern US are infested with invasive species such as *Phalaris arundinacea*, reed canary grass (RCG). A problem with efforts to control RCG is that this aggressive plant usually can recover from various means of control, from seeds in the soil seed bank, from seeds washed in with floods and from difficulty associated with achieving complete kill of the RCG with available herbicides. Active as well as dormant parts underground may not be affected by herbicides. Dormant plant material both on above ground stems and in underground rhizomes is able to resist treatment with commonly used herbicides because herbicides are only effective on actively growing parts in plants (Tu *et al.*, 2001, AquaNeat® label, Fusilade II® label), which implies that dormant tissues cannot be killed. In order to stimulate these dormant parts, plant growth hormones such as cytokinins have been used to activate RCG to releasing its lateral buds from dormancy (Annen, 2010). Annen (2010) suggests that combination of kinetin and herbicide may be an effective means of RCG control. Other studies (Adams & Galatowitsch, 2006) suggest that two seasons

of treatment per year may bring desirable results. There also may be a need to evaluate the efficacy of each of these treatments in a variety of combinations.

### ***Goals and objectives***

A major goal of this study is to improve current methodology for suppression of RCG using herbicides in combination with a plant growth hormone, and testing the effect of different application times during the season. The purpose of this study is to determine which combination of application time(s), type of herbicide, and use of kinetin is best to decrease regrowth of RCG.

The objectives of this study are to:

1. Determine whether or not use of kinetin prior to herbicide treatment will enhance the herbicide effect.
2. Compare the effect of herbicide applied in the fall only, in the spring only and in both spring and fall.
3. Compare the effectiveness of herbicide types based on biomass remaining after treatments.
4. To determine utility of kinetin in releasing dormancy that leads to herbicide susceptibility both in the field and greenhouse.
5. Compare topographically adjacent sites within the study area to find significant effects on the outcome on any of the treatments used.



## **Background**

### ***Reed canary grass***

#### ***Possible methods of control***

Invasive plant populations can be controlled by a number of means, but no ultimate weapon for controlling RCG has been found because different environments may require different treatment plans (Lavergne & Molofsky, 2004). Tested methods of controlling RCG include, seeding with desirable plants (Matthews and Spyreas 2010; Miller *et al.*, 2008; Kim *et al.*, 2006; Perry *et al.*, 2004; Barnes, 1999; Wetzel and van der Valk, 1998), shading (Miller *et al.*, 2008; Iannone and Galatowitsch, 2008; Perry and Galatowitsch, 2006; Mauer and Zedler, 2002), controlled burns (Adams and Galatowitsch, 2006), repeated mowing (Miller *et al.*, 2008; Lyford, 1993), and herbicide applications (Annen, 2010; Miller *et al.*, 2008; Lyford, 1993). Each technique has positive and negative qualities, and it is up to resource managers to decide which is likely to be successful in existing conditions and have lesser adverse effects.

### **Planting seeds for competition**

Post herbicide treatment seeding with desirable plants has been done to increase diversity of an area (Matthews & Spyreas, 2010), to increase ecological services, prevent erosion (Sahramaa & Jauhiainen, 2003) or provide refugia and food for animals (Costanza *et al.*, 1997). Seeding adds desirable native seeds instead of relying on seeds in soil; however, introduced plants, remaining post-treatment, must be able to compete for resources with invasive plants in order to successfully control invasion. Seeding may decrease invasive species by offering competition (Iannone & Galatowitsch, 2008). In Wisconsin, reseeding of 33 native plants following a pretreatment of a grass specific herbicide showed that RCG cover and height was reduced compared to a no treatment control (Wilcox *et al.*, 2007). Invasive plants are usually well-established because they can usually out-compete many kinds of native plants (Lavergne & Molofsky, 2004). The difficulty is finding which mix of plants to use which can compete with RCG likely to be present after initial treatment.

A variety of native and non-native plants have been used to determine their potential to compete with RCG. Barnes (1999) found plants with a mature height of less than 1 m (*Veronia fasciculata*, *Eragrostis pectinacea*, *Panicum virgatum*) cannot compete with RCG, most likely due to shading. Tall plants such as non-native barnyardgrass (*Echinochloa crusgalli*) have been shown to reduce RCG biomass by 65% (Perry *et al.*, 2004), and it appeared to form a competitive cover crop that may have prevented establishment of RCG in a newly restored wetland in Fairborn, Ohio (Amon, 2012, personal communication).

In Minnesota, Iannone and Galatowitsch (2008) tested competitive impacts of American sloughgrass (*Beckmannia syzigachne* (Steud.) Fern.), fox sedge (*Carex vulpinoidea* Michx.), bur-marigold (*Bidens cernua* L.), dock-leaved smartweed (*Polygonum lapathifolium* L.), and Northern willow-herb (*Epilobium glandulosum* Lehm) all mixed with RCG seeds. They found that these cover crops decreased RCG seedling establishment by 89% compared to its original cover. In the local Beaver Creek Wetlands, RCG seems to grow without forming a monoculture in communities containing *Eleocharis erythropoda*, *Carex comosa*, and *Sparganium eurycarpum* (personal observation). Perhaps these species, as well, have some possible utility as competitive species, and should be subject to some research.

### **Shading may be used to control RCG**

RCG can be shaded by taller plants, by dead plants. In Minnesota, Lyford (1993) found that by removing grass clippings after mowing, shading did not affect RCG stem count in treated plots, relative to those where clippings remained, by the end of summer. Kim *et al.* (2006) and Miller *et al.* (2008) described suppression of RCG by two different types of hardwoods that shaded out RCG. Plots with red alder (*Alnus rubra*) and arroyo willow (*Salix lasiolepis*) planted in a RCG monoculture in Washington showed 88-98% cover less RCG than percent cover of control plots 5 months after planting (Miller *et al.*, 2008).

Studies have shown that RCG can shade out river bottom herbaceous plants (Barnes, 1999) and sedge meadows (*Carex* spp.) (Perry and Galatowitsch, 2006; Wetzel and van der Valk, 1998), which makes selecting replacement cover crops complicated. Slower growing plants like *Carex hystercina* (Perry and Galatowitsch, 2006) and *C. stricta* (Wetzel and van der Valk, 1998; Johnson and Zedler, 2012) cannot survive because of RCG's rapid initial growth shades them out. *Calamagrostis* spp. was shown to compete with RCG by Johnson and Zedler (2012) and they both are usually found on sand or silt soils.

#### **Solarization may be used to scorch ground and kill all plants**

Black or sometimes transparent tarps placed over targeted plants can block out light and, by the greenhouse effect, scorch the plants underneath; a process known as solarization. Solarization kills all growing parts and possibly seeds allowing replanting without unintended competition. This type of treatment has proved to be successful by Mauer and Zedler (2002), and Cooke (1997). Mauer and Zedler (2002) used black polypropylene cloth over rhizome fragments and found this treatment decreased RCG above ground biomass. Current restoration projects in tropical wetlands of Costa Rica by Amon (personal communication, 2012) used solarization after mowing. Tarps covered ¼ hectare sites for 4 months after which they are removed and placed on adjacent sites. He found that the solarized area was completely barren with no apparent viable

seeds in soil; therefore, his team planted plugs and seed of locally collected stock after the tarps were moved.

### **Controlled burns as a pretreatment**

Burning can clear the way for more effective herbicide treatment by weakening regrowth of RCG after fire treatment. Burns carried out in the late winter or early spring can also clear dead plant matter that blocks light to soil, and releases inorganic compounds into soil. Adams and Galatowitsch (2006) found that RCG cannot be controlled by burning alone or as a pretreatment to herbicide because of its dense rhizome network underground which is unaffected by the heat of the fire. In fact, burning actually increased RCG shoot density from 520 shoots/m<sup>2</sup> to 1,180 shoots/m<sup>2</sup> (Adams & Galatowitsch, 2006).

### **Mowing to even plant heights and reduce shading**

Mowing is another type of control which can modify growth activity. Mowing may prevent seed formation and increase light to low growing competitive plants. RCG that is frequently mowed will logically have less photosynthetic activity and produces less stored food reserves than un-mowed plants. Mowing to 200 or 300 mm above ground will have no such impact on low growing sedges (Amon, personal communication, 2012), but it affects taller plants. Lyford (1993) found that mowing RCG to 80 mm above ground gave about the same suppression as using a broad spectrum herbicide (glyphosate).

Miller *et al.* (2008) also compared mowing to glyphosate, and found similar results. RCG mowed to 25 mm tall twice in the summer followed by three times in the spring and summer of the second year gave a 72% suppression, but glyphosate treatments in June (first year) and May (second year) had a suppression rate of 89% (Miller *et al.*, 2008). Drawbacks to repeat mowing are that heavy machinery may compact soil and mowers are not always possible to use when area is saturated with water. Mowing prior to seed production may be improbable due to spring rains or inability to access the site.

### **Use of herbicides**

Herbicides are used for control because of their low cost, seemingly fast results, and limited side effects to the environment when applied according to label. Some herbicide formulations can be administered in flooded conditions. There are herbicides that select for either broad leaved plants (usually dicots) or grass species (narrow spectrum), and herbicides that kill both types of plants (broad spectrum) (Tu *et al.*, 2001). Herbicide is usually best when applied to actively growing plants (AquaNeat<sup>®</sup> Label; Fusilade II<sup>®</sup> label). In addition it must be administered under certain permissible weather conditions or seasons, when the herbicide is most effective, when it will not be washed away or when it will not threaten non-target species.

Some herbicides are not EPA registered for use where water is present, which is a concern when treating aquatic or wetland plants. For example, the

label of Fusilade II<sup>®</sup>, states that it cannot be applied over standing water, since under these conditions, herbicide may leach into the ground and contaminate the ground or surface water. However, some herbicides can be applied in parklands when standing water is not connected to streams or other water bodies. In order to decide which herbicide to use, the most important thing to take into consideration is which herbicide type will suppress the undesirable plant without causing too much damage to desirable species.

### **Broad spectrum herbicide with active ingredient glyphosate**

Broad spectrum herbicides, such as those with active ingredient glyphosate, are meant to kill all plants, which makes them useful in killing undesirable monocultures. Glyphosate controls most annual and perennial plants by targeting the enzyme 5-enolpyruvyl 3-shikimate phosphate (EPSP) synthase (Eschenburg *et al.*, 2002). EPSP is a key enzyme in the shikimate biosynthetic pathway that catalyzes the transfer of enolpyruvyl moiety of phosphoenolpyruvate (PEP) to the 5-hydroxyl of shikimate-3-phosphate (S3P) (Steinrücken and Amrhein, 1980; Shaner, 2006; Tu *et al.*, 2001). Glyphosate inhibits synthesis of important aromatic amino acids such as tyrosine, tryptophan, and phenylalanine, needed for protein formation in plants (Carlisle & Trevors, 1988). Glyphosate can also act as a competitive inhibitor of phosphoenolpyruvate to prevent aromatic amino acid synthesis (Tu *et al.*, 2001, Rubin *et al.*, 1984).

Due to presence of other ingredients in the product, some herbicides containing glyphosate are not EPA - registered for use over water according to their respective herbicide labels. These include Round-up® (Monsanto, St. Louis, MO), Razor® (NuFarm Americas Inc., Burr Farm, IL), and Buccaneer® (Tencoz, Inc., Alpharetta, GA). Formulations available that are permissible over water, include Aqua-Neat® (NuFarm Americas Inc., Burr Farm, IL) and Rodeo® (Dow AgroSciences LLC, Indianapolis, IN). These have been found to be nearly harmless to aquatic species when used according to their labels. An advantage of glyphosate is that they have a very short half-life in soil often allowing reseeding or planting within days of their application.

### **Narrow spectrum herbicides for grass species**

Sethoxydim and fluazifop-p-butyl are active ingredients in two different grass specific herbicides (graminicides). Both herbicides kill plants by inhibiting acetyl CoA carboxylase activity, an enzyme needed for formation of essential fatty acids (Page *et al.*, 1994), which in turn inhibits lipid synthesis (Tu *et al.*, 2001). The compromised cell membrane structures eventually cause active growth areas, such as meristems, to collapse and stop growing (Page *et al.*, 1994) thus killing the vegetative part of the plant. Application of sethoxydim and fluazifop-p-butyl is more useful than glyphosate in areas of high plant diversity because it kills grasses, but has no negative effects on broadleaf plants or sedges. Healy and Zedler (2010) found that early application of sethoxydim prevented RCG from flowering which mean no seeds produced that year,



reduced its height by 50%, and reduced its cover to less than 40%. Amon (2012, personal communication) applied sethoxydim in both spring and fall growth phases and found essentially the same thing as Healy and Zedler (2010) and Adams and Galatowitsch (2006), but Amon found that three successive years of treatment were needed for RCG suppression that lasted up to ten years.

### **Application timing**

Timing of treatment relative to the growth cycle may be important. Geiger and Bestman (1990) looked at mobility of glyphosate during different stages of a plant life cycle in sugar beets (*Beta vulgaris*). They found that glyphosate moves with carbohydrates through the phloem of plants; therefore fall application is more effective at targeting below ground plant parts since herbicide moves with carbohydrates to storage in roots. In velvetleaf (*Abutilon theophrasti* medikus), Fuchs and others (2002) found that after 6 hours, glyphosate was readily absorbed by the source. After 30 hours 33% of applied glyphosate translocated to the sink tissues, while 17% remained on the surface of the applied leaf. Most of the applied glyphosate were found in the stem (45%) and the roots (38%). Similar radiolabeled studies found after 24 hours, more than 70% of glyphosate remained on the leaf surface (Camacho & Moshier, 1991). Their conditions in the greenhouse simulated spring application and they recovered 13% of applied glyphosate was transported to the rhizomes in younger (3-4 leafed) plants, and 5% in older (6-8 leafed) plants. Sprankle *et al.* (1975) found similar results in that

younger quackgrass (*Agropyron repens*) absorb herbicide more readily than older plants.

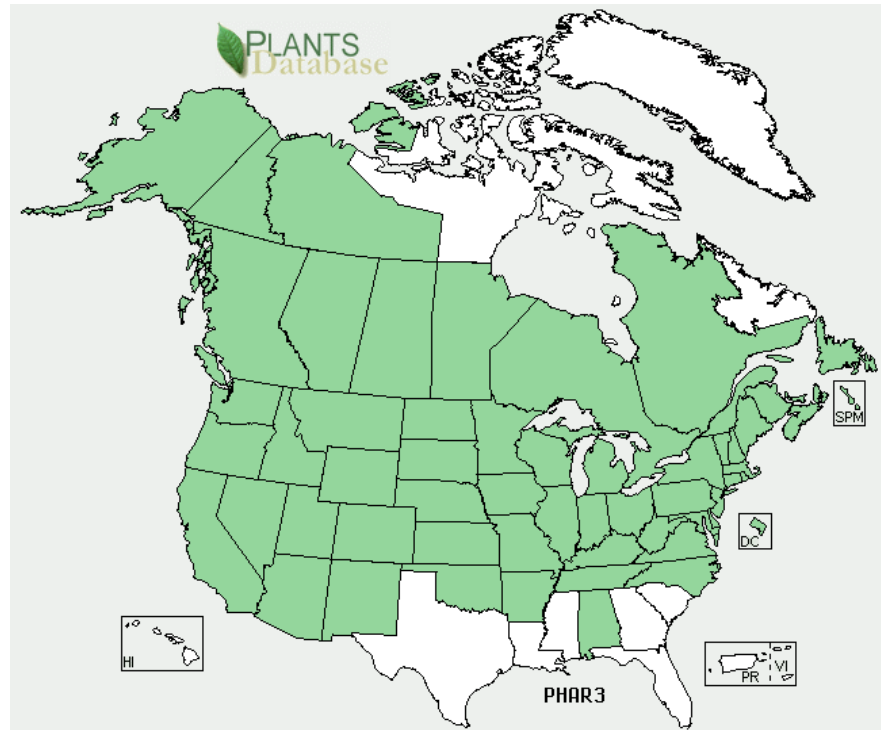
In Minnesota, Adams and Galatowitsch (2006) compared glyphosate applications in late August and late September to mid-May finding that autumn treatments were better than mid-May treatments. Spring spot spraying for 2 years showed 89% suppression of RCG compared to a non-treated control (Miller *et al.*, 2008).

### **Use of kinetin with herbicides**

Kinetin is a cytokinin (a plant growth hormone) that promotes axillary bud growth (Cline, 1994). The stems of RCG have many nodes, each containing dormant meristems that can form buds that develop into new plant culms. Herbicides not effective in controlling RCG may be due to their inability to attack dormant tissue. It was thought that the herbicide may be more effective if dormant meristematic tissue could be activated prior to treatment. Annen (2010) published a study using a cytokinin to release these dormant tissues in order to increase the effectiveness of herbicides. He found little differences in the two graminicides (sethoxydim and fluazifop-p-butyl) in terms of effects on RCG above ground biomass and species diversity when used with kinetin pretreatment. Disking has also been used as a means of releasing dormancy, and kinetin as a pretreatment to herbicide reduced RCG biomass as effectively as disking pretreatment (Annen, 2010).

**Distribution of RCG in the United States**

RCG is present all over the world (Invasive Species Specialist Group, 2010) however; the ISSG does not specify the amount present and if wetland diversity is threatened in all locations. According to the USDA (2012), RCG is considered noxious in Connecticut, Massachusetts, and Washington and is distributed all across North America (Figure 1). In Ohio, it is found in 74 out of 88 counties (USDA, 2012) and is considered a targeted invasive plant by the Ohio Department of Natural Resources (ODNR, 2000). This means RCG is among the plants most difficult to control in Ohio.



**Figure 1 Distribution of *Phalaris arundinacea* from the USDA Plants Database (USDA 2012). Shaded regions in green indicate RCG present.**

### **Impacts of biological invasions**

Invasive species can overtake a habitat and become a near monoculture, reducing plant growth, abundance and diversity of existing plant communities. Diversity is important because a variety of plant species offer different benefits to the environment such as nesting materials, perches for birds, food sources, nurseries, habitats for animals, water purification, soil detoxification, nitrogen fixation, mitigation of greenhouse gases, erosion control, and probably many other benefits we do not know about (Costanza *et al.*, 1997; Sheaffer *et al.*, 2008, Mack & D'Antonio, 1998).

### **RCG benefits and invasive characteristics**

RCG was first brought from Eurasia to the United States by the U.S. Department of Agriculture (USDA) around 1850 to plant in floodplains as a forage crop for cattle (Galatowitsch *et al.*, 1999; Merigliano & Lesica, 1998). However, RCG is difficult for cattle to digest and they prefer not to graze on it (Casler & Jung, 2006). Other expected benefits of RCG included preventing erosion (Sahramaa & Jauhiainen, 2003), removal of excess ammonium and nitrates in soil (Vymazal *et al.*, 2010; Sheaffer *et al.*, 2008), use as a biofuel (Galkin *et al.*, 1997), and paper and fiber production (Hellqvist *et al.*, 2003).

Although RCG may provide some benefits to humans, its spread into both disturbed and high biological value habitats has led to reduced diversity in plant communities (Spuhler, 1994). RCG's aggressive characteristics include having easily dispersed seeds, survival of seeds in soil, and rapid cool season (early) growth. RCG can grow up to 1.96 m (~7 ft.) tall, (Sahramaa & Jauhiainen, 2003), which can easily shade typically slower native sedges and forbs (Perry & Galatowitsch, 2006). RCG not only dominates above ground, but its underground rhizome network is vast, with over 50,000 nodes per 1 m<sup>2</sup> (Maslova *et al.*, 2007). Like most cool season or C3 grasses, RCG's growth is bimodal (Lavergne & Molofsky, 2004). Its early spring growth gives it an advantage over C4 grasses by virtue of its ability to use up nutrients before warm season species are active and RCG's rapidly achieved height shades other plants. Another growth period in fall gives it a second chance to store food to use in spring for rapid aboveground growth. RCG can survive in stressed areas such as in

alkaline environments (Prasser & Zedler, 2010), where high sediment buildup occurs (Kercher & Zedler, 2004), and in flooded conditions (Klimešová, 1995). These aggressive characteristics of RCG allow it to remain dominant through competitive exclusion of native plants.

### ***Hypotheses***

Based on past studies and personal observations, predictions to be tested in this study are:

1. Treatment of RCG with kinetin prior to treatment with herbicides will significantly increase the kill of RCG as determined by biomass.
2. Treatment of RCG with glyphosate will significantly decrease RCG biomass compared to fluazifop-p-butyl.
3. Herbicide application in both spring and fall will reduce RCG regrowth more than a single application.
4. Broad spectrum herbicide will show no significant differences in RCG regrowth compared to grass specific herbicide in reducing RCG percent cover regrowth and percent biomass remaining after treatments.
6. Using double the manufacturer's recommended concentrations of kinetin and fluazifop-p-butyl will significantly reduce amount of RCG stems.
7. The two sites will not yield significant differences in treatments.

## **Materials and Methods**

### ***Site description***

Two study sites were relatively nearby each other (Figure 2) at Phillip's Park in Beavercreek, Greene County, Ohio (39°42'54.36"N, 84°00'48.72"W and 39°42'51.16"N, 84°00'44.47"W) (Figure 3 a-b). One site was on a 2.5% to 10.1% (drops 0.914 m in 9.36 m) downward sloping bank and the other was nearly flat. Both were in the flood plain (100 year flood zone) of Beaver Creek (Figure 2), but the flat site has standing water more often than the sloped site. The flat site was almost a complete RCG monoculture, while the sloped site had about 95% RCG with a mixture of other plants (

This aerial map from Google Earth shows the Phillips Park area. A large portion of the upper half of the image is covered by a red cross-hatched pattern, indicating a specific land use or zoning. Within this patterned area, two points are marked with green pins and labeled: 'Flat Site' and 'Sloped Sites'. Below the patterned area, a dark, irregular shape represents a pond. To the right of the pond, a road is labeled 'Phillips Park Entrance'. The bottom left corner of the map includes a legend for 'Elevation (meters)' with color-coded ranges: 0-25, 25-50, 50-75, 75-100, 100-125, 125-150, 150-175, 175-200, 200-225, 225-250, 250-275, 275-300, 300-325, 325-350, 350-375, 375-400, 400-425, 425-450, 450-475, 475-500, 500-525, 525-550, 550-575, 575-600, 600-625, 625-650, 650-675, 675-700, 700-725, 725-750, 750-775, 775-800, 800-825, 825-850, 850-875, 875-900, 900-925, 925-950, 950-975, 975-1000. The bottom of the image features a scale bar in feet (0 to 1000) and meters (0 to 400), and a north arrow pointing upwards.



Figure 3 a-b. Maps of field site locations.



Figure 3 a. Locations in Dayton, OH, USA.

Field sites are indicated with white box on the map of Ohio (top picture).

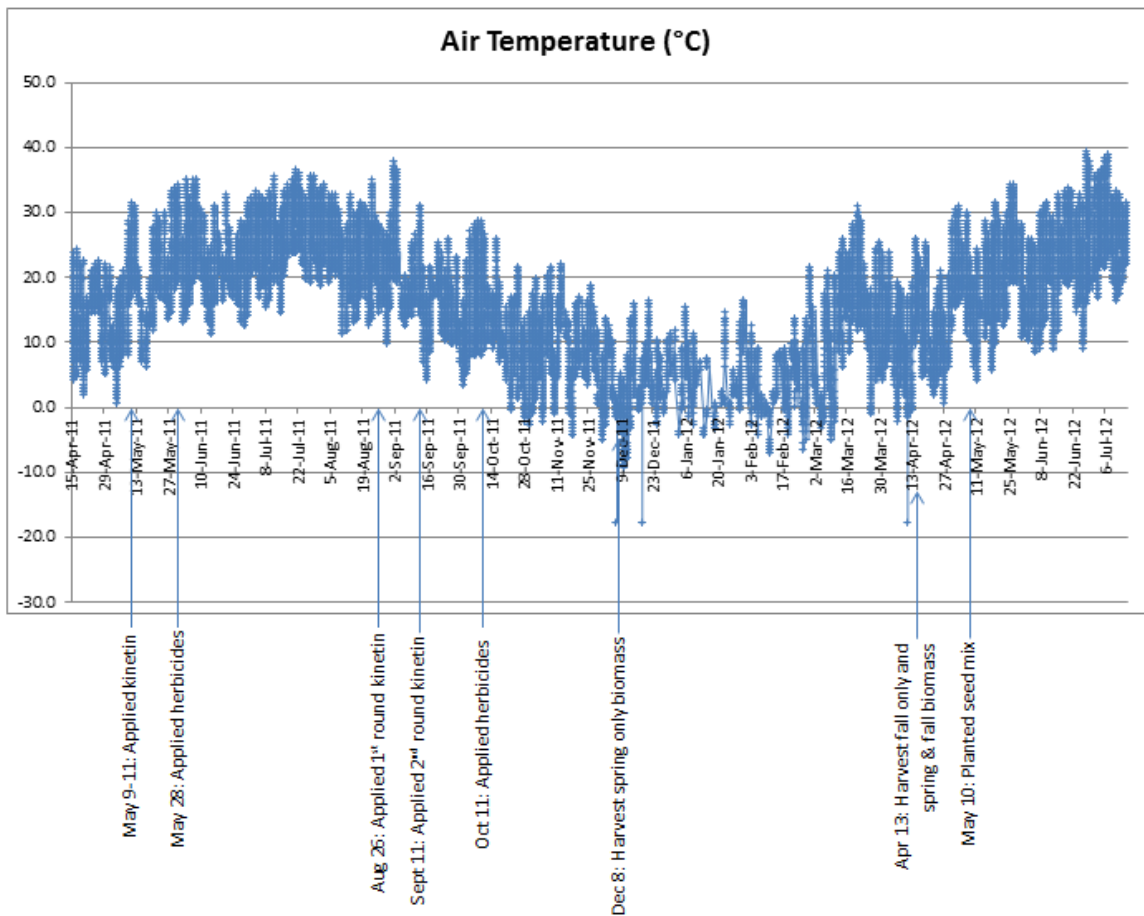
Figure 3 b. Locations in Beavercreek Township, Greene County, Ohio.

Flat site (left;  $39^{\circ}42'54.36''\text{N}$ ,  $84^{\circ}00'48.72''\text{W}$ ) and sloped site (right;  $39^{\circ}42'51.16''\text{N}$ ,  $84^{\circ}00'44.47''\text{W}$ ) on map of Phillips Park (bottom picture).

Arrow points north.

### ***Treatments tested***

The experiments started in the spring of 2011 (Figure 4, Appendix 1).



**Figure 4. Timeline of treatments with daily temperatures in Celsius (y-axis) recorded from the Beaver Creek Wetlands Association monitoring site 150-200 m from the study area.**

Each treatment or treatment combination (n=16) was replicated four times at each site (flat and slope) (Table 1) for a total of 128 treatment plots. There were two application times, one in Mid-May of 2011 and the other in late-August 2011. Figure 4 shows the relationship between experimental action dates and air temperature.

**Table 1. Treatments. Four replicates at each site equals eight replicates of sixteen treatments were used. AquaNeat® (AQN) contains glyphosate, Fusilade II® (F2) contains fluazifop-p-butyl, and X-Cyte™ contains kinetin. Controls used are indicated in the last column. No treatment controls were used to test for effects on using any herbicide, while X-Cyte™ only treatment was used to test if kinetin can increase herbicide efficacy. Note: Only one set of *No treatment* plots were used to compare effects of all seasons.**

Application time	Broad spectrum application	Grass specific application	Hormone application
Spring only	AQN + X-Cyte™	F2 + X-Cyte™	X-Cyte™
	AQN	F2	
Spring and fall	AQN + X-Cyte™	F2 + X-Cyte™	X-Cyte™
	AQN	F2	No treatment
Fall only	AQN + X-Cyte™	F2 + X-Cyte™	X-Cyte™
	AQN	F2	

All treatments, including controls, contained Dawn® dish detergent (1.0%) as a surfactant and blue Turf Mark® (0.78%) in addition to stated treatments. Turf Mark® was a biodegradable dye used to visualize application coverage of treatments. X-Cyte™ (Stoller Enterprises, Inc.®, Houston, TX) was used for the kinetin pretreatment. The broad spectrum herbicide chosen was AquaNeat® (53.8% active ingredient glyphosate in the form of its isopropylamine salt) (NuFarm Americas Inc., Burr Ridge, IL). The narrow spectrum (grass specific) herbicide was Fusilade II® (24.5% active ingredient fluazifop-p-butyl, Sygenta, Greensboro, NC). All stock concentrations and active ingredient concentrations of these agents are shown in Table 2.

**Table 2. Solutions and active ingredients (a.i) used.**

% a.i. in stock as purchased	active ingredient	% of stock used	Short name	final % a.i. sprayed
0.04%	Kinetin	0.04%	X-Cyte™	0.000016%
53.80%	Glyphosate	1.0%	AquaNeat®	0.538000%
24.50%	Fluazifop-p-butyl	0.50%	Fusilade II®	0.122500%

Solutions were mixed according to recommended percentages on their respective labels (Table 3). Exact amounts of stock solution, distilled water (DH<sub>2</sub>O), Turf Mark®, and Dawn® dish detergent used were based on a need of about 9.0 L of solution (Table 3). Solutions were applied using a 4 gallon backpack sprayer to cover leaves, but short of dripping off the plant.

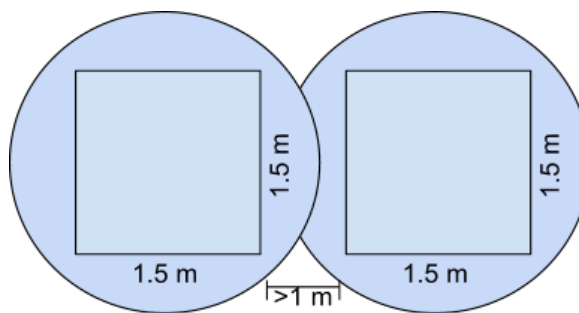
**Table 3. List of solution contents and the date prepared for spraying. AquaNeat® = glyphosate, Fusilade II® = fluazifop-p-butyl, and X-Cyte™ = kinetin.**

Application date	Application site	% of stock	Solution	Stock (mL)	DH <sub>2</sub> O (L)	Turf Mark® (mL)	Dawn® (mL)
09-May-11	flat site	0.04	X-Cyte™	3.752	9.38	73.16	93.80
11-May-11	sloped site	0.04	X-Cyte™	2.504	6.26	48.83	62.60
28-May-11	both sites	1.00	AquaNeat®	90.00	9.00	70.20	90.00
28-May-11	both sites	0.50	Fusilade II®	45.00	9.00	70.20	90.00
26-Aug-11	both sites	0.04	X-Cyte™	3.60	9.00	70.20	90.00
11-Oct-11	both sites	0.50	Fusilade II®	45.00	9.00	70.20	90.00
11-Oct-11	both sites	1.00	AquaNeat®	90.00	9.00	70.20	90.00

Fusilade II® is not approved for use where surface water was present, according to its label. With the study sites being in a wetland, extra caution must be taken with Fusilade II®, which is toxic to fish, since it can leach into ground water or run off in to streams (label, Sygenta; Tu *et al.*, 2001). AquaNeat® has insignificant side effects and is approved for use over water (label, NuFarm Americas, Inc.). The wetland sites chosen were seasonally dry and only rarely inundated with water, making control of the RCG in dry seasons possible.

### ***Quadrat layout***

The quadrat was approximately 1.5 m x 1.5 m (actually 5 ft. x 5 ft.). Plots were deliberately sprayed slightly beyond each quadrat into 1 m buffer zone to eliminate edge effects (Figure 5). Quadrats on the sloped sites were positioned on available space within the areas dominated by RCG and also to avoid shading and allelopathy from walnut trees. Treatments at both sites were randomly assigned to an area in a grid using a random number generator (Appendix 2 and Appendix 3).



**Figure 5. Application to avoid edge effects. Squares indicate 1.5 m x 1.5 m quadrats. Circles indicate spraying application area. Shaded areas show application coverage.**

### ***Kinetin pretreatment application***

Pretreatment with tested whether if kinetin can release dormancy and significantly increase herbicide efficacy.

### **Spring application**

Mid to late April (optimal treatment time) rains prevented kinetin application until early May. Spray nozzle on backpack sprayer was calibrated to apply kinetin at a rate of 1 pint/acre (1.2L/ha) (short of dripping off the plant) on the flat site spring treatments on May 9, 2011 from 8:30pm-10:00 pm under dry (61-68°F/16.11-20°C), low wind conditions (1.0-2.5 mph/1.6-4.0 kph), while the sloped site was sprayed at the same rate on May 11, 2011 at 6 pm-7pm under dry (47-52°F/8.3-11.1°C), low wind conditions (1.8-5.4 mph/2.9-8.7 kph). By the time of application RCG was 0.76-1.2 m (2.5-4 ft.) tall, dark blue-green in color, but not ready to bloom. These were not the same conditions of Annen's (2010) application because my RCG was tall and near full maturity. He treated them when RCG had either three or four leaves.

### **Fall application**

Both sites for fall treatment were treated with kinetin short of drip point on August 26, 2011 (wind: 1.0-3.9 mph/1.6-6.3 kph, temperature: 77.7-82.0°F/25.4-27.8°C). Because plant nodes did not sprout after the first application, sites were retreated again on September 11, 2011, 6pm-9pm (wind: 0.2-1.0 mph/0.32-1.6 kph, temperature: 67.0-70.1°F/19.4-21.2°C). Plant nodes not sprouting new culms indicated their dormancy was not broken. For all treatments, at least two hours of exposure was accomplished before rainfall. According to label info on X-Cyte™, this time period is sufficient for the desired activity.

## ***Grass specific compared to broad spectrum application***

### **Spring application**

For spring treatments, herbicides solutions shown in Table 3 were applied short of dripping off the leaf on May 28, 2011 under low humidity, with slow winds (0-6.7 mph/ 0-10.8 kph) at 29-34°C (70.8-78.9°F/21.6-26.1°C) from 1pm to 8pm. RCG at this time had a height of 4-5 ft. (1.2-1.5 m) which was close to its maximum height and just short of flowering; therefore, it was in a slow growth stage.

### **Fall application**

Fluazifop-p-butyl for fall treatments on both sites was applied at 8 am (wind: 0.4-0.7 mph/0.64-1.13 kph; temp: 48.9-50.3°F/9.4-10.2) on October 11, 2011 under slightly moist conditions due to morning dew. Glyphosate was applied the same day at 5 pm (wind: 3.3-6.2 mph/5.31-9.97 kph, temp: 75.7-77.1°F/24.3-25.1°C) under dry conditions. At this time, RCG was in its second growth spurt and either growing from seeds from plants not treated in the spring, or from rhizomes, so there was a mixture of heights.

**Data collection**

Percent cover data of dominant and sub dominant living plant species were recorded monthly starting the day after herbicide treatment until RCG went dormant for winter. Final percent cover data was taken 12.5 weeks after herbicide application to measure regrowth before spraying for fall treatments. Percent cover of live plants after fall treatments were taken 4 weeks after application, and again after winter on April 12 (a day before spring harvesting).

Quadrats made of 1 inch diameter PVC pipes were fashioned into about a 1.524 m x 1.524 m (really 5x5 ft.) square for determining limits of vegetation measured for percent cover and biomass. The quadrat was used to delineate, the area of the percent cover per plot. Percent cover was estimated to the nearest 5% (20 cover categories) by one person in order to keep interpretation consistent.

Since all plants were dead, it was impossible to differentiate which plants were killed by the herbicide and which went dormant for winter, all above ground RCG biomass from mid-May treatments was harvested on December 11, 2011. RCG treated in both spring and fall and in fall only was harvested in mid-April 2012. Care was taken to harvest only green material that represented un-killed or regrown material. Electric hedge trimmers and serrated knives were used to cut stems at soil-plant interface  $\pm$  20-30 mm above soil.

December harvested biomass (0.001 g – 2,000 g) was spread out on newspapers in a dry barn until grass was air dry to the touch (at least 6 weeks).



April harvested grass was dried in paper bags at 80°C for at least 48 hours to establish a constant dry weight. All large non-RCG matter such as sticks and tree leaves was removed before processing. After preliminary dry weight was recorded, grass was milled in order to homogenize sample. A portion of the total dry weight, greater than 3.0 g of the milled sample was placed in 51 mm x 62 mm aluminum weigh dish at 80°C for at least 48 hours in an oven until constant dry weight to evaporate all water from samples. Out of 48 samples, four replicates from 12 random samples were used to determine that the milling successfully homogenized the sample with less than 1% standard deviations. After dry weight was recorded, the samples were ashed in a muffle furnace too burn up all organic material using Hoskins (2002) loss on ignition (LOI) procedure with the exception of using different temperatures and containers. The difference between the dry mass and ashed mass would show the amount of organic matter present in each sample. This method omits water, soil, and sediment deposits collected during harvest. A lower ashing temperature was used because the melting point of aluminum is 660.4°C, but my temperature tests showed that aluminum weigh dishes used can withstand a maximum of 500°C, at which some dishes had minor melting on the bottom. A temperature of 480°C was the temperature used so that aluminum would not become too soft to handle. Ash free dry mass (biomass) was calculated by measuring the amount of organic material (LOI) present in each harvest.

### ***Data interpretation***

Species richness and live percent cover data was initially assessed before any treatment. Percent cover data taken from spring and fall treatments were used to compare differences amongst timing of applications. Four factors analyzed were the timing of application, use of kinetin, herbicide type, and site type to determine interaction effects and effectiveness among types of treatments using four-way factorial ANOVA on Statistical Analysis Software: S.A.S version 9.2.

Since biomass was a destructive measurement, it could only be taken once to quantify regrowth after treatments. After plants became dormant for winter, plots were harvested on December 8, 2012 for analysis of all RCG above ground biomass in spring only treatment. Harvest included any RCG present regardless of whether it appeared live, dead, or dormant. During summer, herbicide treated grass died and decayed before fall harvest; therefore the fall harvested biomass represents new growth. New growth could be from a number of sources but it did not appear to be from seed germination based on field observations. The material harvested for biomass determinations probably grew from either subterranean rhizomes or rhizome that grew from untreated grass at the perimeter of the sample plot. Fall only and spring & fall treated plots were harvested on April 13, 2012 for actively growing above ground biomass. This harvest included material grown after vernalization during early spring 2012. The double treatments sprayed in spring and fall of 2011 had an extra growing period in June through November 2011, while the fall only plots had regrowth only in early spring 2012.

Since measurements were taken during different times and represent different types of regrowth, the RCG biomass was normalized to percent of grass remaining (PGR) by averaging the untreated controls of each harvest time in both sites and using Equation 1 and Equation 2.

**Equation 1. Spring biomass PGR.**

$$\text{For spring biomass measurements, } \text{PGR} = 100 \left( \frac{\text{spring biomass}}{914.758} \right)$$

**Equation 2. Fall biomass PGR.**

$$\text{For fall biomass measurements, } \text{PGR} = 100 \left( \frac{\text{fall biomass}}{262.510} \right)$$

PGR was used to compare effectiveness of kinetin use, herbicide type, site type, and timing of application (both number of applications and comparison of single fall or spring application) using a four-way ANOVA. All ANOVA analyses were run using a model p-value of  $p < 0.0001$ . After an initial comparison, the model was re-run using only significant variables (defined by p-value). Tukey's honest significant difference (HSD) test with family wise significance was used to determine if means are significantly different in specified variables.

## ***Supplemental studies to field experiment***

### **Reseeding**

Initial visual assessment of the two sites used showed a near monoculture of RCG. It is unknown what types of seeds are in the soil, if any. Reseeding may have been necessary to fill in bare areas in order to compete with RCG seeds from growing after herbicide treatments. For plots that were recently harvested and presented bare soil in which to sow seeds (treatment in fall only and spring and fall total 80 plots), sixteen seeds (two of each species) of *Carex comosa*, *C. cristatella*, *C. frankii*, *C. hystericina*, *C. vulpinoidea*, *Eupatorium perfoliatum*, *Scirpus atrovirens*, and *S. cyperinus* were each stratified on April 10, 2012 in separate sandwich sized zip-lock bags with washed sand. The seeds were kept moist at 5°C for four weeks to break dormancy. Sixteen seeds total were spread over half of each 1.5 m x 1.5 m plot on May 10, 2012, while the other half of the plot was used as a control to ensure plants which grew were from the seed mix planted.

### **Greenhouse study**

Since there is little literature on kinetin effects with fluazifop-p-butyl on RCG, a more controlled test in the greenhouse was designed to determine whether or not the fluazifop-p-butyl and kinetin concentrations could be improved. We also wanted to determine if application of kinetin and herbicide and a younger age would increase the effect of herbicide. Four concentrations of fluazifop-p-butyl and four concentrations of kinetin (Table 4) were tested with five

replicates of each treatment. RCG plant rhizomes with apparently active culms were taken in mid-January from a local wetland near study site location and grown in ~101.6 mm (really 4 inch) pots in a greenhouse (23°C) for 30 days in order for them to acclimate to the environment. Kinetin at three different concentrations (Table 4) was applied to cover leaves just short of dripping off the plant on February 22, 2012 when plants were about 150 mm tall. Fluazifop-p-butyl was applied 2 weeks later when sprouting of lateral buds was observed. Stem heights of living (green) RCG, number of alive (green) stems, and general observations were recorded monthly starting two weeks after herbicide application. Yellow and brown stems were noted as dying and dead stems respectively, but their counts were not used for statistical analyses.

To determine what happens to the rhizomes in the field after herbicide treated plants, at the conclusion of the experiment when herbicide treatments displayed a complete kill, all above ground material was cut at the soil-stem interface and removed to see if regrowth might occur. To observe regrowth success, two replicates of each treatment type (32 plants) were then placed incubated at less than -0°C to simulate plant life cycle during winter months for two weeks, another two replicates of each treatment type (32 plants) were cut at the soil-plant interface to simulate mowing, and one set of replicates of each treatment type (16 plants) was dug up to observe underground rhizomes and roots. The cold treated plants were returned to the greenhouse after two weeks to observe effects. The underground rhizomes from the last replicate were discarded after observation because rhizomes were destroyed during harvesting

process. A two-way ANOVA was performed on the absolute change in number of stems per pot. Tukey's HSD test with family-wise level of significance of 0.05 was used to determine statistically significant differences between concentrations of kinetin and herbicide.

**Table 4. List of treatments used in greenhouse study to analyze the effects of halved or double recommended concentration of Fusilade II® (fluazifop-p-butyl) and X-Cyte™ (kinetin). \*Bolded font Denotes recommended concentration. Each treatment was replicated five times.**

	Fusilade II® 0%	Fusilade II® 0.25%	<b>*Fusilade II® 0.50%</b>	Fusilade II® 1.0%
X-Cyte™ 0%	K <sub>0</sub> F <sub>0</sub>	K <sub>0</sub> F <sub>0.25</sub>	K <sub>0</sub> F <sub>0.50</sub>	K <sub>0</sub> F <sub>1.0</sub>
X-Cyte™ 0.02%	K <sub>0.02</sub> F <sub>0</sub>	K <sub>0.02</sub> F <sub>0.25</sub>	K <sub>0.02</sub> F <sub>0.25</sub>	K <sub>0.02</sub> F <sub>0.25</sub>
<b>*X-Cyte™ 0.04%</b>	K <sub>0.04</sub> F <sub>0</sub>	K <sub>0.04</sub> F <sub>0.25</sub>	K <sub>0.04</sub> F <sub>0.25</sub>	K <sub>0.04</sub> F <sub>0.25</sub>
X-Cyte™ 0.08%	K <sub>0.08</sub> F <sub>0</sub>	K <sub>0.08</sub> F <sub>0.25</sub>	K <sub>0.08</sub> F <sub>0.25</sub>	K <sub>0.08</sub> F <sub>0.25</sub>

## **Results**

An initial plant assessment shows the sites to be near monocultures of RCG with a lower richness compared to after one herbicide treatment. Flat site had a higher richness of five different species compared to three species on the sloped site (

Appendix 5).

***Response of RCG to treatment measured by percent cover***

Two weeks after herbicide application in spring, herbicide treated RCG plots were apparently completely dead. All above ground vegetation was discolored. No samples of below ground material were taken to check for viable rhizomes. Fluazifop-p-butyl took double the amount of time (2 weeks) to turn plants chlorotic compared to glyphosate (1 week). Out of the four factors tested, site type and herbicide type showed a strong interaction (



Appendix 7). In most cases glyphosate reduced RCG percent cover more than fluazifop-p-butyl (Figure 6,

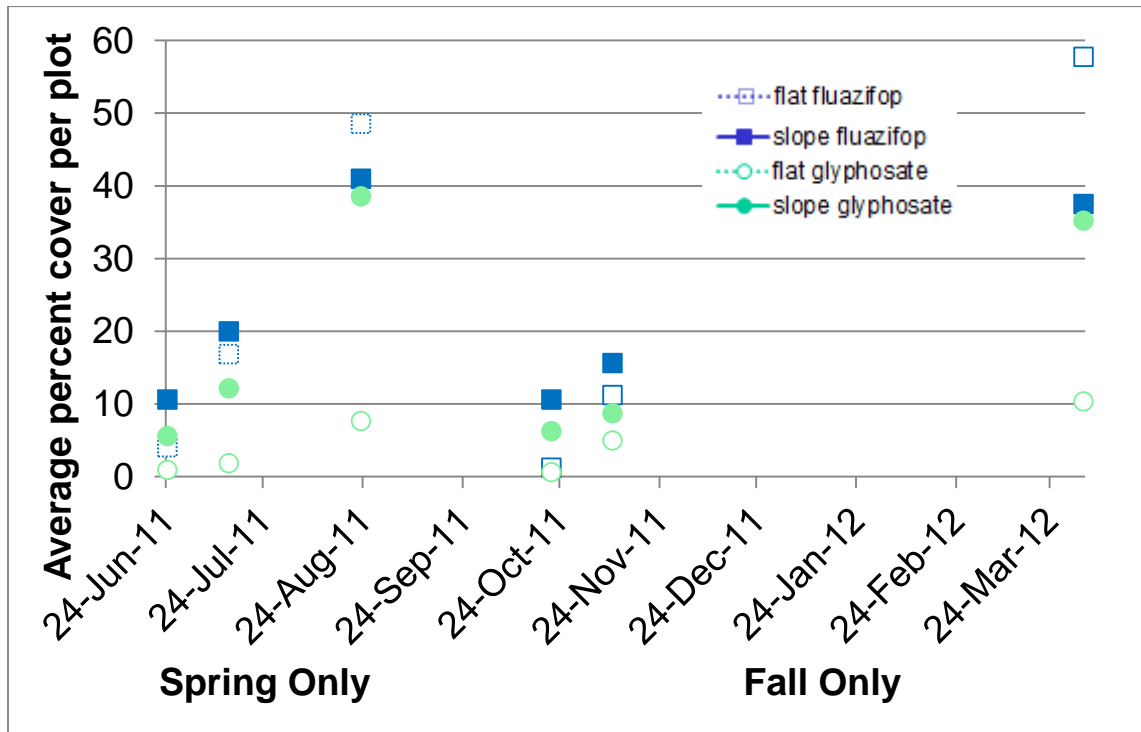
Table 5). Time of application ( $p=0.9976$ ), kinetin use ( $p=0.4006$ ), and site type ( $p=0.3817$ ) did not affect herbicide efficacy, therefore figure 5 does not include information on kinetin (

Appendix 7). There was a significant interaction between site and herbicide type ( $p < 0.0001$ ), which means the two herbicides performed differently on the two different sites. Differences among herbicide type may not be the same for both flat and sloped sites. Treatments on the two sites, for the

most part, did not show statistically significant differences. The only exception was glyphosate on the flat site. Glyphosate treatment on the flat site had significantly lower percent cover regrowth, compared to using the same herbicide on the sloped site (

**Table 5).** For full statistical report, see

Appendix 9.



**Figure 6. Average percent cover of RCG from June 24, 2011 to March 24, 2012 shows distinct site and herbicide differences after herbicide treatment. Controls were constantly at 100% cover (Figure 6), and therefore were omitted from this figure. Spring & fall double treatment was not significantly different than fall only treatment, and therefore was also omitted. See**

**Table 5 for average standard deviation and Tukey groupings.**

**Table 5. Comparison of treatment and site combinations ranked in order of decreasing percent covers (number of observations (n) =24). Same letters preceding treatment combination indicates no significant differences between of their means (Tukey's HSD,  $p < 0.0001$ ).**

<b>Treatment Combination</b>	<b>Mean</b>	<b>Standard Deviation</b>
A. Control/Flat	99.79%	1.021
A. Control/Slope	93.33%	19.597
B. Fluazifop-p-butyl/Flat	51.63%	34.020
B. Fluazifop-p-butyl /Slope	39.83%	25.380
B. Glyphosate/Slope	37.46%	35.268%
C. Glyphosate/Flat	8.58%	5.356%

Fluazifop-p-butyl showed more variability than glyphosate (Figure 7) and appears to be half as effective (45.729% fluazifop-p-butyl 23.021% glyphosate). Among the different herbicide treatments (control, glyphosate, and fluazifop-p-butyl), each differ significantly from each other according to the Tukey HSD test ( $p < 0.0001$ ; Figure 7).



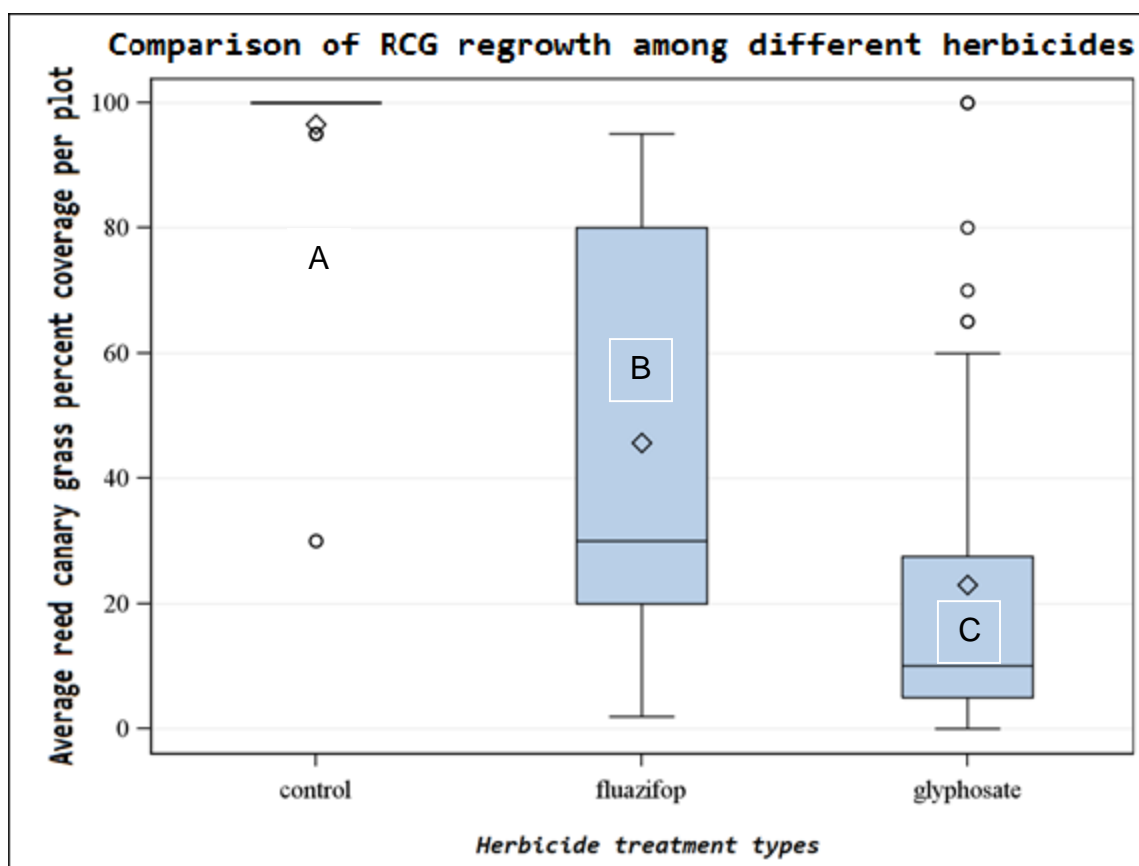


Figure 7. Comparisons of live RCG regrowth quantified by average percent covers (n=48) measured before plots were harvested. Site type, kinetin use, and application times were omitted since they were not significantly different ( $p>0.05$ ). Diamonds denote mean; circles denote outliers; lowest horizontal line denotes minimum; highest horizontal line denotes maximum; boxes show upper and lower 25% quartiles; horizontal line inside box denotes median. Same letters indicate means were not significantly different (Tukey's HSD,  $p<0.0001$ ).

***Species richness assessment of spring only treatment***

Species richness of the two sites and treatment within sites was reassessed after one spring treatment on August 10, 2011. Both sites had increased in richness after spring herbicide treatment (

Appendix 5 and Appendix 6). Before treatment, the sloped site had a lower species richness compared to the flat site (

**Appendix 5).** The flat site still had a higher richness of eight different species compared to the six species on the sloped site (

Appendix 5 and Appendix 6) with new plants emerging such as poison hemlock (*Conium maculatum* L.), bindweed (*Convolvulus arvensis* L.), giant ironweed (*Vernonia gigantea* L.).

After spring treatment, previously unseen plants sprouted in areas previously dominated by RCG (

Appendix 5 and Appendix 6). RCG treated with herbicide appeared to be dead and did not continue to sprout for the second growing period in fall. Untreated RCG outside the plots pushed down from winds into treated areas re-rooted from living stems and were probably responsible for some of the biomass measured there and were included in the measurements. There were no signs of RCG sprouting from seeds or from most of the below ground rhizomes which suggested that all parts of the RCG plant was killed. Later experiments suggest that the spring treatment may have not killed the rhizomes. There were a few patches of RCG from rhizomes, which may have been missed when treating with herbicide.

After fall treatment, no regrowth of other plants were seen most likely because plants were going dormant for winter. For its next growth period in April 2012, spring and fall treated plots showed RCG were not complete monocultures and were less dense compared to the previous year. In fall only plots, RCG was still the dominant plant, but on the sloped site, poison hemlock cover paralleled RCG percent cover. On the flat site, Canada thistle and common ragweed became the 2<sup>nd</sup> and 3<sup>rd</sup> most dominant plant after RCG in terms of percent cover.

Glyphosate treated plots had more non-RCG plants the following growing period after treatment compared to fluazifop-p-butyl treated plots. Fluazifop-p-butyl treated plots had more RCG regrowth. After spring treatment, more non-RCG plants were seen compared to measurements taken after fall treatment, which indicates that herbicides can give other plants the opportunity to grow if

RCG was eliminated. Plants such as green headed coneflowers (Figure 8), dogbane (*Apocynum cannabinum* L.), and wingstem sunflower (*Verbesina alternifolia*) were present, but no plants from the reseeding experiment sprouted. Plants such as poison hemlock (*Conium maculatum* L.) became more prevalent in the sloped site and Canada thistle (*Cirsium arvense* L.) became more prevalent in the flat site after herbicide treatments. RCG was still the dominant species.



**Figure 8. A stand of green headed coneflowers over 5 feet tall out-competing RCG (about 1.5 foot tall) after treatment with glyphosate in both spring and fall 2011. Photo credit Jim Amon, July 14, 2012.**

The dense stand (Figure 8) of Greenheaded Coneflowers (*Rudbeckia laciniata*) was atypical of the regrowth but suggested that growth of native stands of non-invasive plants can replace RCG. That site was treated with glyphosate in both growing periods but failed to kill the susceptible *R. lacinata*, probably because the site was treated before the plant was in active growth in the spring and after it became dormant in the fall.

***Response of RCG to treatment measured by percent biomass of green material remaining after treatment***

The value for percent grass remaining may be misleading in that it was based on the biomass at the time of harvest. It did not represent percent kill because dormant subterranean parts of the plant were not measured. Control provided the baseline of unaffected material that grew during the growing season before harvest, or in the case of spring biomass measures its new growth in spring. In treated plots the biomass measured represents remaining green and or regrowth. Most of the sites sprayed with herbicide appeared to have little or no live material above ground within a month after treatment. Subsurface testing was not performed to see if there was living material there.

Percent Grass Remaining (PGR) collected during April 2012 represents both unaffected RCG and RCG regrowth from previously unseen sources. PGR collected in December 2011 is both killed RCG and dormant RCG biomass (refer to Appendix 1 for dates of treatment and harvest).

**Site type**



Site type did not significantly influence PGR ( $p=0.4577$ ). It appears that there is less variability among PGR on the flat site compared to the slope site (Figure 9). Differences in site affected herbicide efficacy on PGR. Sloped sites however had a consistently lower PGR mean when treated with fluazifop-p-butyl compared to the flat site (Figure 9). Statistically, the sloped site, fluazifop-p-butyl (36.003% PGR) performed as well as glyphosate (31.144% PGR;  $p=0.2262$ ); however, on the flat site, glyphosate (14.318% PGR) was more than three times as effective as fluazifop-p-butyl (49.627% PGR).

### **Treatment type**

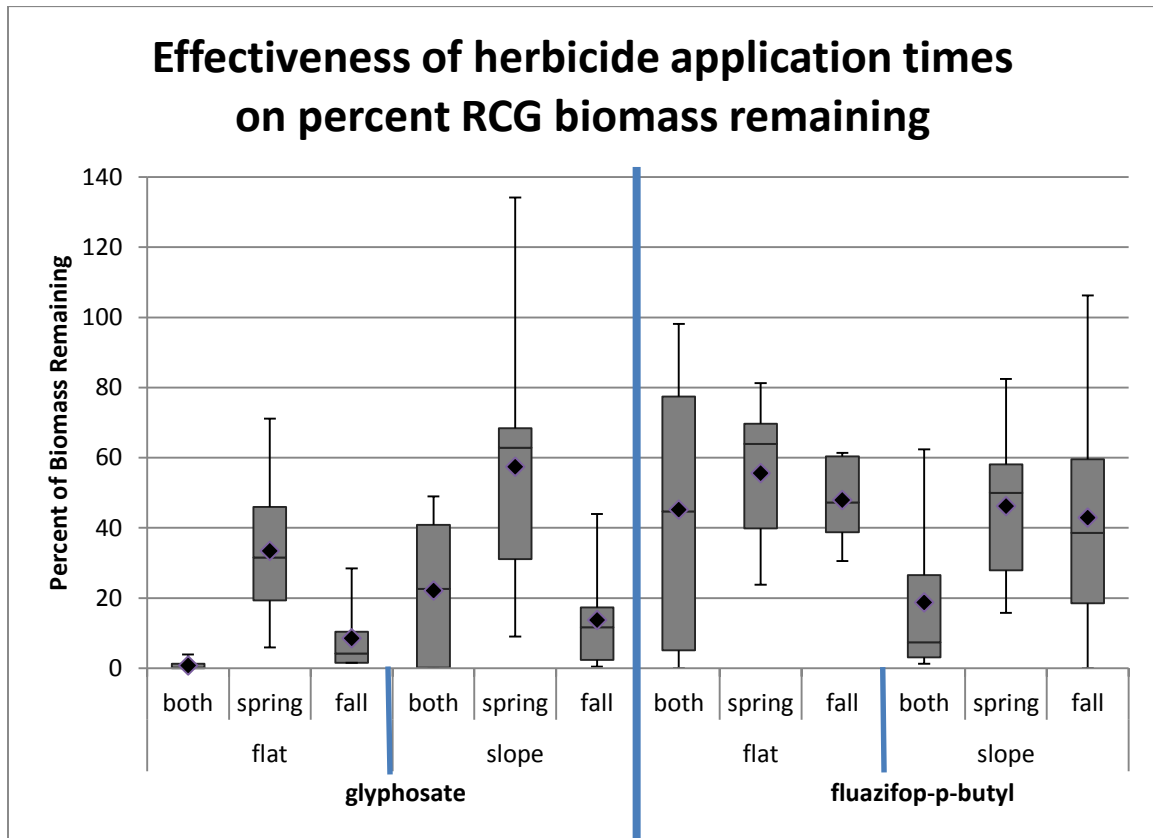
Kinetin did not significantly influence PGR ( $p=0.7435$ ) regardless of herbicide type. Herbicide type had the greatest effect on PGR ( $p<0.05$ ). Glyphosate was more effective at reducing PGR compared to fluazifop-p-butyl with the exception of application in spring only on the slope site (Figure 9). The variability was higher in fluazifop-p-butyl treatments compared to glyphosate with the exception of spring only treatments.

### **Applications of treatments**

Application time, and number of applications had a great effect on PGR ( $p<0.05$ ). There was a clear difference between timing of applications with the exception of fluazifop-p-butyl application on the flat site (Figure 9). Treatments in fall following spring treatments showed the greatest reduction in PGR (21.781% PGR). Treatments in both spring and fall were consistently lower in PGR compared to single treatment in fall or spring, with the exception of fall application of glyphosate on the sloped site (Figure 9). Fall application (28.342%

PGR) appeared to be almost twice as effective compared to spring application (48.197% PGR). Figure 9 shows that fall only application PGR means were consistently lower than spring only application. For full statistical report, see

Appendix 9.



**Figure 9. Comparison of percent RCG above ground biomass remaining after harvest in December 2011 and April 2012 (n=48). Kinetin use was omitted since it was not significantly different ( $p=0.7435$ ). Diamonds denote mean; horizontal line of box denotes minimum; highest horizontal line of box denotes maximum; boxes show upper and lower 25% quartiles; horizontal line inside box denotes median.**

### ***Reseeding and regrowth after biomass harvest***

When RCG biomass was harvested on April 13, 2012, there were many bare plots from apparently killed RCG. These bare plots implied that after RCG was killed, there were few viable seeds or plants in soil seed bank. Thirty days after planting on June 10, 2012, no sign of the eight types of plants were seen in any of the plots. The lack of plants was most likely due to lower amount of rain (Figure 10), or higher temperatures (27-35°C daily; Figure 11) compared to the previous year which was typical of Ohio climate, and seeding with too few seeds to account for potentially low germination rates.

The sites was rechecked on October 11, 2012 for presence of the eight types of seeds planted to determine if fall growing conditions were more favorable to seed germination. None of the species planted were present; however plants such as non-native Canada thistle (*Cirsium arvense* L.) were seen periodically throughout the flat site. Presence of native wingstem sunflower (*Verbesina alternifolia*) was seen as the second most dominant plant in the sloped site, with RCG being the most dominant. Dense mats of RCG were present near the water on the sloped site, while near the pathway further south on the site, bare areas were seen with little RCG. A year after treatment, sites treated with glyphosate showed little differences compared to fluazifop-p-butyl. Fluazifop-p-butyl treated plots had more RCG regrowth compared to plots treated with glyphosate.

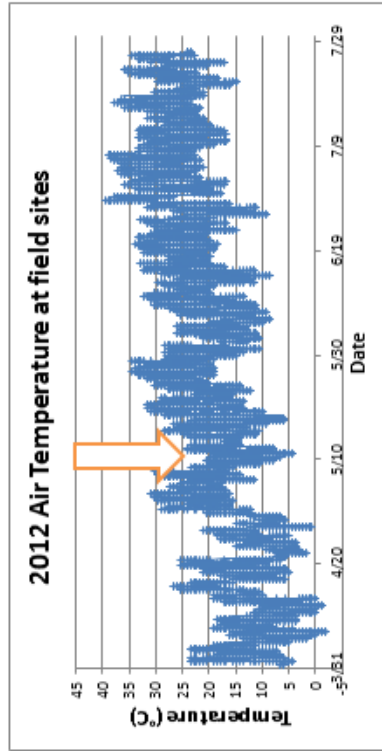
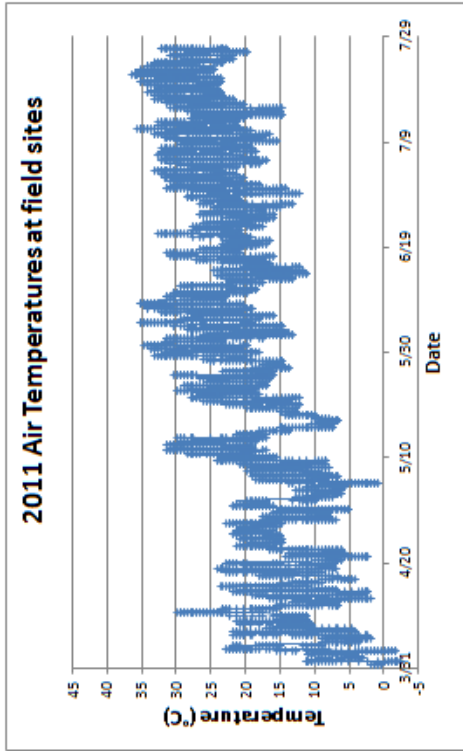


Figure 11. Comparison of temperature at field site during reseeding from April 1- July 26 of 2011 and 2012. X-axis shows time, while y-axis shows temperature in Fahrenheit. Notice temperature on y-axis in 2012 is higher compared to 2011. Arrow indicates when seeds were planted.

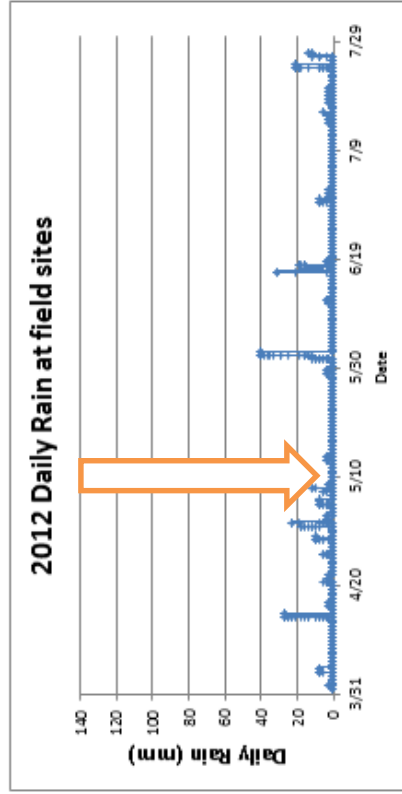
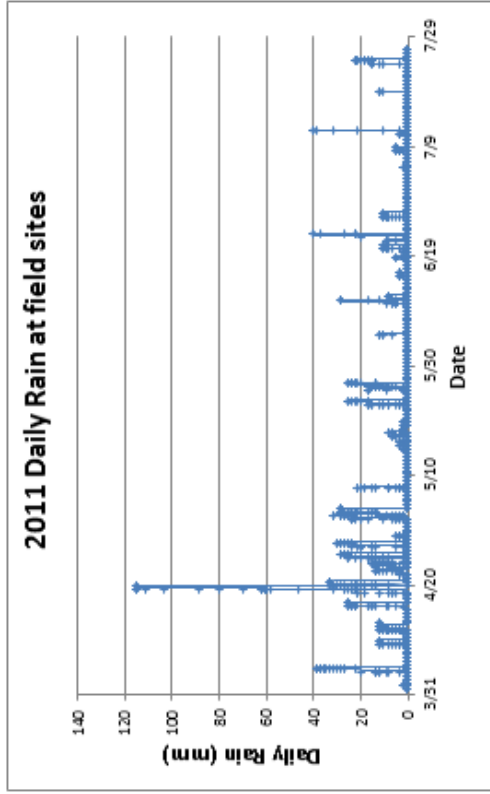


Figure 10. Comparison of April 1- July 26, 2011 (top) and April 1- July 26, 2012 (bottom) average rainfall at field site recorded from the BCWA monitoring site 150-200 m from the study area. The y-axis shows inches of daily rain, and the x-axis shows date. During reseeding in May 10, 2012, rainfall lower compared to previous year. Arrow indicates

### ***Greenhouse study***

To ascertain whether the concentration of kinetin applied in field study was within the correct concentration range, a greenhouse study was undertaken. Labeling on the hormone provided by Stoller USA, recommended 0.04% of stock (X-Cyte™) would be best, but conversations with a scientist at the manufacturer suggested further testing should be performed because the manufacturers had no experience using the cytokinin with RCG. RCG plant rhizomes with growing buds were collected in mid-January from a local wetland near study site location and grown in ~101.6 mm (4 inch) pots in a greenhouse for 30 days in order to acclimate.

All concentrations of kinetin were successful at stimulating dormant buds in nodes to sprout. As in field trials, herbicide treatments showed an apparent 100% kill after 51 days from initial herbicide treatment (negative values indicate loss of green stems in

Table 6). As in field studies, kinetin as a pre-treatment did not enhance effects of the herbicide ( $p=0.2376$ ), meaning there was not a strong interaction between the change in number of living stems among different levels of fluazifop-p-butyl and kinetin. Significant differences final stem count mean were seen in the different concentrations of fluazifop-p-butyl ( $p<0.0001$ ) and kinetin ( $p=0.0017$ ) (Table 7). Fluazifop-p-butyl treated plants had a significantly lower stem count (Tukey's HSD  $p=0.05$ ) compared to the untreated control; however, there were no differences in plant death when using 0.25%, 0.5% or 1% fluazifop-p-butyl product.

Another way to interpret results as that without exposure to the herbicide, the kinetin induced more of the dormant nodes to become active. Thus, at the end of the experiment the herbicide treated plants had less green sprouts than those not treated. Compared to pots with no kinetin the greatest stimulation of sprouting was with 0.02% kinetin product. The concentration of herbicide that caused the most rapid kill of stems was most obvious at days 34 and 44 of the experiment (

Table 6). Without kinetin the loss of viable culms (at 0.25% herbicide) was similar to no kinetin and 0.08% kinetin suggesting that the kinetin at 0.08% was no more effective than non-treatment. At the mid-concentration of herbicide, loss of green stems was similar at all concentration of kinetin except 0.04%, but the trending of data suggests that the lower loss there was an anomaly. The highest concentration of fluazifop-p-butyl quickly reduced the green stem count in non-kinetin treated and at 0.02% and 0.04% kinetin product. Again, trending of data suggest that the result with 0.08% kinetin and 1% herbicide was anomalous.

In combination with herbicide, the recommended concentration of X-Cyte™ (0.04%) had the largest loss of stems; while 0.02% kinetin product was the least effective. Using Tukey's HSD test ( $p=0.05$  significance), we found some transformed means had overlapping means (Table 7) meaning 0.02% and 0.08% were not significantly different than the 0% control, but there was a difference between 0.02% and 0.08% kinetin product. Kinetin treated plants

were observed to have a lower maximum height than no treatment control plants, which is an observation also seen in the field for some plots.

**Table 6. Mean percent change in number of living stems since application of kinetin. Herbicide concentration is in columns, while hormone concentration is in rows.**

			Fusillade II® (fluazifop-p-butyl) Concentration (%)			
			Day	0	0.25	0.5
X-Cyte™ (kinetin) Concentration (%)	0	34	80.0	6.7	33.3	26.7
		44	143.3	-73.3	-93.3	<b>-100.0</b>
		64	210.0	<b>-100.0</b>	<b>-100.0</b>	<b>-100.0</b>
	0.02	34	126.7	16.7	30.0	20.0
		44	213.3	-53.3	-90.0	<b>-100.0</b>
		64	290.0	<b>-100.0</b>	<b>-100.0</b>	<b>-100.0</b>
	0.04	34	63.3	6.7	6.7	11.7
		44	110.0	-60.0	-33.3	-93.3
		64	140.0	<b>-100.0</b>	<b>-100.0</b>	<b>-100.0</b>
	0.08	34	90.7	-20.0	-6.7	-1.7
		44	125.7	-73.3	-86.7	-68.3
		64	186.0	<b>-100.0</b>	<b>-100.0</b>	<b>-100.0</b>



**Table 7. Transformed means of change in stems. Post-hoc test with X-Cyte™ (n=20). Tukey's HSD test with family-wise level of significance = 0.05 with the same letter in Tukey grouping are not significantly different. Notice some means (0% and 0.08%) fall into two groupings.**

Tukey Grouping		Transformed Mean	X-Cyte™
	A	1.31008	0.02%
B	A	1.26104	0.00%
B	C	1.07563	0.08%
	C	1.03292	0.04%

Kinetin appears to have stimulated growth of RCG apparently from above surface and subsurface release of dormant tissue. The largest increase in stems was with 0.02% kinetin. Regardless of the concentration of either kinetin or fluazifop-p-butyl all above ground parts of RCG were dead by day 64. Seventy days after kinetin treatment and 54 days after fluazifop-p-butyl treatment, underground parts of the pots were examined by viability. Visually, rhizomes seemed to be alive and after cold vernalization (<0°C, two weeks) all produced growing shoots indicates they were not dead, but had become dormant. Since unvernalyzed control did not resprout, it is apparent that the cold treatment is a key part of the long term resistance of RCG to fluazifop perhaps the other herbicides.

Field experiments treated in the fall that experienced vernalization over winter had regrowth that may have come from dormant underground stems as seen in greenhouse experiments. Field experiments treated in spring but analyzed by fall collection of biomass would not be expected to show regrowth

because exposure to cold was not encountered. Unfortunately, I did not examine the treatment plants after either spring or fall treatments to see if rhizomes spouted in spring but not fall.

## **Discussion**

The goal of this study was to determine which combination of application time, kinetin use, and herbicide type is most effective in reducing RCG regrowth. In addition, I sought to determine if site topography is a significant factor in reducing RCG regrowth.

### ***Herbicide effectiveness was not uniform across sites***

The sites were in close proximity (within the same park), but they both exhibited differences in drainage rates, elevation, plant diversity, amount of shade, and seed types in soil bank. Plots on sloped site were scattered near the creek, as opposed to the plots on the flat site being in a grid-like fashion in one open area. The sloped site was at a slightly higher elevation and show faster drainage into the nearby creek compared to the flat site. Sloped sites also exhibited more diversity, while the flat site had small patches of plants spread throughout. More shading from nearby trees was scattered about the sloped site, while the flat site may only have shading on the outer edges of the grid.

ANOVA analysis of percent grass remaining (PGR) showed that there was an interaction between site type and herbicide type, which means that some herbicides may be more effective depending on characteristics of the site. RCG percent cover and PGR trends showed glyphosate on the flat site to have lower regrowth compared to glyphosate plots on the sloped site. These varying results

due to site show the importance of incorporating environmental characteristics into management plans.

While it is not known why site physical characteristics affect the results, the major difference may be related to drainage and the physiological status of the plant brought about by amount of and duration of water saturation in the soil. Oxygen deficits in saturated soil might mean the plants on the flat site are under oxygen stress that makes them more susceptible to herbicide (Mitsch & Gosselink, 2007). Movement of oxygen to oxygen deprived roots may carry herbicides with it.

***Kinetin as a pretreatment to herbicide showed same suppression as herbicide only treatments***

Kinetin use was thought to increase herbicide efficacy by decreasing amount of dormant, non-herbicide sensitive, parts of RCG. However, kinetin had no effect in terms of biomass or percent cover on control or treatments with either herbicide. In the field and greenhouse, kinetin only treatments appeared to be visually shorter compared to untreated plots. In the field there were no significant differences with kinetin use found in fall or spring harvested biomass. This may be because cytokinins stimulate lateral growth as opposed to apical growth (Cline, 1994). Kinetin may not have been applied at an ideal time because application was delayed due to spring rains (Figure 10). During the time of application, the RCG was at least 1.25 m tall and near its maximum height. Like herbicides, kinetin is more effective when applied during periods of rapid growth

because this is when the plant is actively transporting nutrients and photosynthates throughout the plant.

In greenhouse study, all RCG stems were killed eventually, regardless of herbicide concentration. The dormant nodes along the stem were also dead and did not re-spout during the duration of the experiment. Some combinations of kinetin and herbicide worked faster than others. The recommended concentration, 0.04% X-Cyte™ showed the highest loss of stems when used with any herbicide concentration, and the only concentration which had significantly different results compared to the 0% control. Half the recommended concentration (0.02% X-Cyte™) may have been too dilute, as it had comparable results to the 0% control. Double the recommended concentration (0.08%) also showed effects comparable to 0.04% X-Cyte™, so there is no need to waste stock solution for the same result.

The activity of kinetin in these controlled experiments suggests that it can provide additional help in controlling RCG, but results from the field suggest that variable field conditions make use of this pretreatment unreliable. The plants in the greenhouse experiment were selected in winter so their behavior was equivalent to springtime growth. In field experiments, fluazifop-p-butyl applied during the spring had the least reduction in biomass and that may reflect non-transport to roots and rhizomes. Further supporting that idea is the lack of death noted in the rootstocks of greenhouse treated plants.

Other differences between the field study and the greenhouse study are that the smaller RCG were sprayed with kinetin in the greenhouse where there was a greater chance to assure complete coverage. In the field, there were dense mats of RCG, so it is possible that parts of the plant could have been missed. The field sites had taller RCG (at least 1.5 m) with more nodes along the stem, while the RCG in the greenhouse were sprayed when plants were 8 inches tall with zero to two dormant nodes.

Annen's study (2010) found his kinetin pretreatment, when coupled with Sethoxydim E Pro (a.i. sethoxydim) or Fusilade DX (a.i. fluazifop-p-butyl), decreased RCG biomass by at least 50% compared to using only herbicide. His success with kinetin may be due to his biomass sampling time. He treated RCG in the spring and harvested biomass in the fall. The treated RCG may have been dormant. Additional observations the following year would be beneficial to determine the success of retarding RCG regrowth long term. Another difference between my study and Annen's was the time kinetin was applied. He applied one round kinetin in late May to early June (RCG was at 2 to 3 leaf growth stage) and a second round of kinetin twelve days after, while my treatment only had one round of kinetin application (RCG in 3 to 5 leaf stage). Annen used two rounds of kinetin because McIntyre (1971) observed that kinetin effects on lateral bud outgrowth wore off after 12 days. He gave no reason to believe that the initial treatment was not 100% successful. I hypothesized that one round would be enough since once all dormant parts are stimulated; herbicide would be effective on those areas. Herbicide treatments appeared to kill all RCG in applied areas

after four weeks. However, RCG regrew from either seeds or rhizomes from nearby untreated plots. Annen treated a bigger area and subsampled within his plots. In the field, RCG from untreated areas blew over and re-rooted. Another difference between our studies is that Annen applied herbicide (RCG at 3 to 4 leaf stage) five days after kinetin, while I waited until RCG's lateral buds were seen which took at least two weeks (RCG in 5 to 8 leaf stage). Since herbicides work most effectively when the plant is rapidly growing, according to their labels, Annen might have applied herbicides at an optimal time to transport kinetin to underground portions of the plants. A problem in spring 2011 was there were frequent rains which delayed kinetin application and in turn herbicide application, so RCG was more mature and, perhaps, not growing rapidly. In addition, temperatures were higher than usual that season. RCG is a C3 grass which prefers to grow in cool, moist soil (Sahramaa and Jauhiainen, 2003); therefore RCG may not have been rapidly growing because of these high temperatures. My kinetin application was delayed and had to be applied when RCG was 0.76-1.2 m (2.5-4 ft.) tall, which seems to be when growth is slowing down as it nears its maximum height (~2m). According to Annen (2010), RCG growth in Wisconsin grows slower than RCG in Ohio. He applied kinetin when RCG had two to three leaves, and then after 12 days, RCG was in the three to four leaf stage. Amon recorded observations at various sites with RCG in Dayton, OH on April 18, 2013 and found that RCG stage may vary in rates of growth heights and leaf stage (

Appendix 8), thus treatment may be sensitive to local climate. It may be impossible to apply kinetin and herbicide at the stages Annen applied his treatments because RCG in Ohio grows at a faster rate than Wisconsin.

***Fall application reduces biomass more than spring application***

Percent remaining biomass showed that fall application was more effective than spring application. For fall treatments, live RCG regrowth was harvested in spring the year after vernalization, therefore its biomass is mostly from rhizomes that were not killed by the herbicide treatments. For spring treatments, biomass was harvested when plants became dormant for winter, which means its biomass represents RCG regrowth from seeds or rhizomes not affected by herbicide treatments. RCG regrowth measured in percent cover showed there were no significant differences between treating in the spring compared to the fall, most likely due to a high amount of variability in fluazifop-p-butyl treatments. A higher treatment area 5 times my sampling plot would have lowered the variability. Unlike biomass harvesting, percent cover is not a destructive measurement, therefore data can be taken repeatedly to increase observation number. Percent cover is a subjective measurement based on an estimated amount with respect to each quadrat. It is difficult to take into account density and height, which are characteristics that contribute to biomass.

After spring herbicide applications, seeds were not present in seed heads which indicates spring sprayings can decrease RCG regrowth from seedlings.



Fall herbicide treatments may have been more effective than spring treatments because herbicide may have been transported with carbohydrates to its roots in the fall more-so than in the spring. That effect is noted by Geiger and Bestman (1990), who used sugar beets to find that glyphosate moves with manufactured sucrose to roots for storage before going dormant for winter. The importance of spring application seems to be two-fold. First it seems to prevent formation of seeds that would have formed if a fall only approach is taken, and second it appears to give an extensive kill of the grass. If that is the case, competitive species may be able to grow and shade which may suppress regrowth from seeds that germinate or from rhizome not killed. This observation concurs with the preliminary findings by Amon (personal communication, 2011) that repeated fall and spring treatment of actively growing RCG over a period of three years were able to suppress the invading grass for a period of at least ten years. Amon postulated that once RCG was suppressed, competitive flora created shade that inhibited re-establishment of new RCG seedlings. Fall application would be used in cases, where spring application was not possible due to flooded site conditions or other problematic spraying conditions.

In Minnesota, Adams and Galatowitsch (2006) tested four treatment plans for RCG. They sprayed a broad spectrum herbicide (glyphosate) in mid-May, late August, and late September, and found one late August or late September treatment was just as effective in reducing RCG biomass as two treatments in mid-May. These results suggest that transport is equal during different times of

the year. My results suggest that while a single fall treatment is better than a single spring treatment, application both times is more effective.

Geiger and Bestman (1990) could not have anticipated a two season effect because the plant they used, sugar beets, is not a two growing season plant. The key to herbicide activity is application during the rapid growing period (Howard, 2012, personal communication; Fusilade II<sup>®</sup> label; AquaNeat<sup>®</sup> label). One active growing period is typically in April and May, and the other is in September. Average daily temperatures in those periods are from 18-24°C (64-74°F) high to 6-12°C (43-53°F) low (Current Results, 2012). In May or June where Adams and Galatowitsch (2006) and Annen (2010) made their treatments in Minnesota and Wisconsin, they probably had temperatures of 20-26°C (68-78°F) high to 9-14°C (48-58°F) low (Current Results, 2012). These temperatures are roughly equal to the fast growth temperatures for RCG I noted here in west central Ohio from early April to May (Figure 4).

Wisconsin Reed Canary Grass Management Working Group (2009) compiled a variety of suggestions based on past research. They suggested that a broad spectrum herbicide in late summer will maximize translocation to the roots. It is unknown if fluazifop-p-butyl is also translocated to the roots as effectively as glyphosate based on published studies. I have found that in the greenhouse, after a single grass specific herbicide application (simulated in spring conditions) and a vernalization cycle, RCG regrew from its rhizomes,

which means the herbicide did not completely kill the underground meristematic tissue.

***Two treatments suppress RCG better than single treatment***

Since spring plus fall treatment gave the least amount of remaining RCG one might posit that one potential difference is in the recovery of rhizomes that have survived a single treatment (as noted in greenhouse). Two treatments have the potential to kill the newly grown plants and those plants have little food in reserves, perhaps making them more susceptible to a second herbicide treatment.

Adams and Galatowitsch (2006) tested single broad spectrum herbicide (glyphosate) application in mid-May or late August/September compared to application both seasons. Similar to my results, they found two applications in mid-May and late August/September are better at reducing RCG biomass than one application. Annen and others (2005) found that in west central Wisconsin, one grass specific herbicide application (sethoxydim) at the end of May (68-78°F/20-26°C high, 48-58°F/9-14°C low) showed no difference in reduction of RCG biomass compared to spraying at the end of May and early August. They found a 50% decrease in RCG with grass specific herbicide, Vantage (sethoxydim). I found that single application compared to two application treatments showed no differences in terms of percent cover, but in terms of percent RCG biomass remaining, two treatments each year was better than a

single treatment a year. Spring application prevents seed formation and thereby decreasing RCG the following growing period. Fall application may transport herbicides with carbohydrates to underground storage, where it is toxic, and thereby decreasing RCG regrowth from rhizomes (details in previous section on spring and fall application). A double treatment would eliminate both methods of regrowth. After spring treatment, there were no seeds in the seed head of the treated RCG. Regrowth also came from RCG in untreated plots falling over and re-rooting from meristematic tissues along stems. Regrowth after fall did not come from seedlings, but mainly from rhizomes. During the harvest in fall, no visible seedlings sprouted.

A key differences in my study compared to the study by Annen (2010), is that they mentioned that dead mats of RCG may have obstructed second round of application. I took extra care to lift mats of both alive and dead RCG to ensure an equal coverage to spring application. Another difference was the type of active ingredient (sethoxydim) used for the grass specific herbicide; however, another study by Annen (2010) showed little differences between sethoxydim and fluazifop-p-butyl.

***Glyphosate is more effective in reducing RCG regrowth than fluazifop-p-butyl***

Grass specific herbicides, such as fluazifop-p-butyl, are preferable as a means to control grass invasion in wetlands since they do not kill sedges or broad leaved plants and because early spring application avoids damage to C4 plants that have not sprouted yet. However, in most RCG scenarios, the invasive plant has taken over a habitat and became a monoculture. In near monoculture situations, we tested if a grass specific herbicide could reduce RCG resurgence compared to one broad spectrum herbicide, glyphosate.

In all forms of measurement, it was clear that both herbicides significantly reduced the amount of RCG compared to a no treatment control. A percent cover assessment of live RCG and percent RCG biomass remaining showed that plots on the flat site sprayed with glyphosate had a significantly lower percent cover compared to the fluazifop-p-butyl making it the most effective form of treatment. However, glyphosate on the slope site did not have the same effect. The percent cover trends of glyphosate treatment on the slope site were comparable to fluazifop-p-butyl treatments on either site. The high success of glyphosate treatment might be because the flat site was a near monoculture with desirable seeds or rhizomes in soil. After all plants were killed with glyphosate, green headed coneflowers rhizomes were able to grow. The sloped site lacked native non-invasive seeds in soil and was mostly dominated by poison hemlock that grew after glyphosate treatment on the sloped site along with RCG, which in turn decreased the percent cover of RCG. In addition, the Wisconsin Reed Canary Grass Management Working Group (2009) recapitulated past

publications to conclude that grass specific herbicides should not be applied when RCG is over a 0.3 m (1 foot) tall, while glyphosate can be applied at higher RCG heights (Camacho and Moshier, 1991). The delay in herbicide application due to weather caused me to apply herbicides when RCG was about 1.2 m tall. In management, a broad spectrum herbicide spot treatment may be necessary. In the case of poison hemlock and Canada thistle treatment with 2,4D (specific to broadleaved plants) could be used and still allow desirable grasses and sedge to flourish. Reseeding may be another important follow-up step after treatment to promote establishment of desired and RCG competitive species.

Glyphosate consistently produced a lower average regrowth in terms of percent cover and biomass than fluazifop-p-butyl. The mobility of glyphosate has been well studied (Geiger and Bestman, 1990; Marquis *et al.*, 1979; Fuchs *et al.*, 2002), however, little is known about fluazifop-p-butyl distribution in grasses. Fluazifop-p-butyl was observed to have a slower kill. In the field, fluazifop-p-butyl takes twice as long as glyphosate to turn the plant yellow. In the greenhouse, under 0.25% concentration, which is half the recommended strength, plants treated with fluazifop-p-butyl were shown to take at least 50 days to completely turn yellow. This lower concentration eventually killed the top growth of the plant and using the lower concentration may have provided an opportunity for maximum transport though out the plant. This slower uptake of fluazifop-p-butyl may show that the herbicide is not being translocated the same way glyphosate is. Since the plant with dead above ground culms were shown to have viable

underground parts it is apparent that transport to the rhizome and roots as seen in glyphosate is not occurring.

Since fluazifop-p-butyl has not been used as long as glyphosate, it is not as well studied. The greenhouse study shows that the optimal concentration of fluazifop-p-butyl appears to be 0.25%, since 0.5% and 1.0% were shown to do just as well as the lower concentration. The label on the herbicide recommends closer to 0.5% perhaps because field use can't provide the thorough coverage that can be achieved in the greenhouse. For future experiments, the concentration used in the field study could be lowered to 0.25% to see if it works as well as 0.5%. Another greenhouse experiment could be to examine survival of roots and rhizomes after glyphosate application. Roots and rhizomes were not killed with fluazifop-p-butyl; therefore it would be interesting to determine if glyphosate can be transported underground and kill the roots.

After herbicide treatments in the spring, RCG sprayed within the plot grew from nearby RCG that had fallen over and re-rooted in the study plot. This shows that spring herbicide application may prevent growth from rhizomes during RCG's second growing period, which may allow other plants to grow. It also emphasizes the importance of treating all nearby RCG plants. A similar observation was seen in the greenhouse. After herbicide treatment, all RCG turned yellow/brown and did not re-sprout. Furthermore, the cold treated plants following the greenhouse study showed that RCG can still sprout the following growing period, which shows the need for repeated yearly treatments. This

observation was also seen in the field during spring harvest of fall treated plants. The green matter harvested was from rhizomes and not from RCG seedlings.

Regrowth of plants as in Figure 8 was unexpected since the plot shown with green headed coneflowers was treated twice with broad spectrum glyphosate. It appears to be a pure stand, so diversity had not changed but a non-invasive species replaced the invasive plant. The green headed coneflowers were able to survive the glyphosate most likely because application occurred when they were dormant. Since they are a “wetland plant” and may grow where Fusillade II® is prohibited, AquaNeat may prove to be a good option in wetlands despite it being a broad spectrum herbicide assuming seeds or dormant rootstocks or rhizomes are present. RCG was still present in the plot, although at a much smaller percent cover and lower height, which shows that there are plants which can out-compete RCG if given the right opportunity. In addition to green headed coneflowers, dogbane, wingstem sunflower, and ragweed continued to grow after glyphosate treatments. This finding indicates that subsurface seed or other plant propagules can and did survive the non-selective herbicide strategy.



### ***Reseeding after herbicide treatment failed to establish native plant species***

Reseeding in mid-May showed no success, most likely due a combination of factors. In the weeks immediately after replanting, the sites received a low amount of rain compared to the previous year, especially during the month of April in 2011. The average temperatures were higher in 2012 than in 2011. These dry conditions and high temperatures may cause the vernalized seed, with no capacity to remain dormant, to germinate and then die for lack of water. Observations in October 2012, after the seeds had cooler temperatures and more rainfall, still showed no signs of the eight types of plants, which support the theory that the seeds are no longer viable. In future experiments, reseeding during a cooler time, preferable with period of rainfall may increase germination rate. Another alternative would be to plant dormant seeds around late February or early March, therefore the seeds can germinate when conditions are preferable. The idea of stratifying the seeds was to increase germination rates and that would be good if sown in cool, wet spring conditions. Planting immediately after herbicide is sprayed in spring would decrease competition from RCG for the following growing period in fall. Stevens and Fehmi (2011) found a high rate of success at reducing invasive Buffelgrass (*Pennisetum ciliare*) when older, native Arizona cottontop (*Digitaria californica*) plants were established immediately following a disturbance. This indicates that the best time to plant these seeds would be after RCG harvest.

Since locally collected seeds have a 50% germination rate of seeds from a nursery (Wisconsin Reed Canary Grass Management Working Group, 2009),

more seeds per 1.5 m x 1.5 m plot area may increase success of growth. The original plan of using only 16 seeds of each species per half plot was based on germination of at least 50% of the seeds planted, and the average sizes of species planted. The goal was to shade the RCG, and not for the chosen seeds to shade out each other. Reseeding done by Healy and Zedler (2010) were also unsuccessful but they also noted annual reestablishment of RCG that undoubtedly competed with seed applied.

Many practices have been attempted to control RCG but no universal methods has proven to be broadly successful. Adams and Galatowitsch (2006) found burning had little effect except to reduce the seed bank. While they found glyphosate treatment to be somewhat effective, recolonization from seed, or suboptimal timing of glyphosate treatment made herbicide treatment a poor method. Certainly, their results suggest that burning (or anything that reduces seed production) combined with glyphosate may be more successful. My result suggest that spring treatment with herbicide (fluazifop or glyphosate) will reduce seed bank of RCG and the second treatment with herbicide enhances the removal of RCG.

Timing of herbicide application may be problematic (Annen, 2010; Adams and Galatowitsch, 2006; Healy and Zedler, 2010). Annen suggests herbicide application at a 3 to 4 leaf stage but that is rarely possible to accomplish in large tracts of land due to flooding, rain, temperature too low for herbicide action and manpower. Using two times (or more) of application (Adams and Galatowitsch,

2006; Healy and Zedler, 2010) as I have done, may increase herbicide efficacy and multiyear treatments are probably necessary for control (Healy & Zedler, 2010). Healy and Zedler (2010) also suggest that many further influence the outcome of any control method that *adaptive management* needs to be applied to control RCG.

My results, like those of Healy and Zedler (2010) found that below-ground rhizomes were graminicides resistant. This again, suggest that multiyear treatments will be needed to control RCG.

## **Conclusion**

The purpose of this study is to determine which combination of application time(s), type of herbicide, and use of kinetin is best to decrease regrowth of RCG. Kinetin application in the field was not shown to affect herbicide efficacy. In my field sites, spraying glyphosate in early spring and fluazifop-p-butyl in the fall both without kinetin seemed to be the best method to suppress RCG. Broad spectrum herbicides application in early spring should occur when RCG is one of the few actively growing plants; therefore, sedges will not be affected by the herbicide. Spring is most likely to present problems of flooding, rain or standing water on the site. AquaNeat® or other formulations approved for use in aquatic conditions could be used. Fall is more often dry and could allow use of fluazifop-p-butyl. If the spring treatment killed RCG and permitted suppressed species to grow, fluazifop would not harm those non-grass species and has the advantage of killing only RCG.

An early treatment of glyphosate in spring in monotypic RCG stands would allow other plants to compete and sprout in the fall. While fluazifop was in general, less effective than glyphosate it did reduce the biomass to about 50% of the controls and in one location it was reduced by about 80%. However, fluazifop-p-butyl selectively kills only grass while glyphosate kills all active plants.

Thus fluazifop-p-butyl leaves the sedges and broad leaved plants to compete for resources in an area where they initially have little competition.

Spring spraying may not be possible due to rainfall or budget concerns. If only one spraying is possible, a single treatment with glyphosate would be advised in the fall. The effects of glyphosate were significantly different at the two sites despite these plots being in close proximity. This shows that other factors such as shading, drainage, and types of seeds in soil can aid in designing a management plan. A single treatment with fluazifop-p-butyl in the fall reduced RCG biomass to about 50%. Unlike glyphosate, fluazifop can give non-grass species a chance to grow with less competition.

Use of either herbicide during the two growing periods, fall and spring, was generally more effective, but for unknown reasons the flat site was not as responsive under fluazifop treatment. Fluazifop treatment, while inferior to glyphosate has the advantage of saving any non-grasses present and would be most appropriate where numerous non-grass species are mixed with the RCG. While RCG tends to form monocultures early parts of an invasion may be more diverse and would be appropriate targets for fluazifop rather than glyphosate. Since it is recognized that some plants like *Sparganium eurycarpum* and *Carex comosa* seem to reside in stable association with RCG, those sites could be treated with fluazifop followed by over seeding in an attempt to increase diversity.

It is evident that either treatment type is often less than 100% effective or multiple research papers (Adams & Galatowitsch, 2006; Hall & Zedler, 2010;

Healy & Zedler, 2010; Lavergne & Molofsky, 2004) have said that RCG reestablishment is a major problem. Based on that observation it may be wise to plan multiple year treatments, each of which would incrementally reduce the coverage by RCG. Since rain and flooding events can often interfere with herbicide application the knowledge that either fall or spring treatments are partly effective provides a management strategy of spraying as whenever possible in fall, spring, or preferably in both growing periods.

Since I observed different effects of the same treatment on nearby sites, land managers should take into account the conditions specific to their site before implementing a management plan. Plant species richness and diversity within the treated area can determine whether a broad spectrum herbicide (1% glyphosate) or a grass specific herbicide (0.25% fluazifop-b-butyl) is appropriate.

RCG maturity level and metabolic activity are important indicators of when to apply herbicides. According to their respective labels both herbicides should be applied when grasses are young and rapidly growing (about 0.5 m tall). At this time, plants are actively metabolizing nutrients or building cell walls to elongate their apical meristems. However, there is little experience with RCG in the literature and the range of plant height that will produce the best kill is not certain. Applying herbicides when plants are shorter decreases the amount of herbicide needed to cover their leaves and treats them when they are most probably metabolically active. Since so many observations, mine and published,

suggest or imply that multiyear treatments may be needed, I posit that such an approach is all but mandatory.

A land manager must be attentive to the site both before and after implementing a management plan because undesirable seeds may sprout after RCG is killed. In these cases, supplemental spot spraying of plant specific herbicides may be necessary before replanting the area with desirable plants either by seed or plant plugs. Without the competitive exclusion offered by replacement planting, seed carried by flood or animals will rapidly recolonize RCG. Therefore any management program must employ replacement species.

RCG is a big problem across North America because of its aggressive characteristics. The principles outlined in this study do not just apply to RCG, but it may help suppress other invasive grass such as *Phragmites* spp.

### Literature Cited

- Adams, C. R., & Galatowitsch, S. M. (2006). Increasing the effectiveness of reed canary grass (*Phalaris arundinacea* L.) control in wet meadow restorations. *Restoration Ecology*, 14, 441-451.
- Amon, J. P. (2010-2012). (D. L. Fong, Interviewer)
- Annen, C. A. (2010). Prospects for disrupting rhizome apical dominance prior to chemical treatment of *Phalaris arundinacea*. *Ecological Restoration*, 28, 291-299.
- Annen, C. A., Tyser, R. W., & Kirsch, E. M. (2005). Effects of a selective herbicide, sethoxydim on reed canarygrass. *Ecological Restoration*, 23, 99-102. Retrieved December 9, 2012, from <http://er.uwpress.org/content/23/2/99.full.pdf+html?ijkey=6a06fca6ea7ce0e0a930cc6418606a01c11435f9>
- Barnes, W. T. (1999). The rapid growth of a population of reed canarygrass (*Phalaris arundinacea* L.) and its impact on some riverbottom herbs. *Journal of the Torrey Botanical Society*, 126, 133-138.
- Camacho, R. F., & Moshier, L. J. (1991). Absorption, Translocation, and Activity of CGA-136872, DPX-V9360, and Glyphosate in Rhizome Johnsongrass (*Sorghum halepense*). *Weed Science*, 39, 354-357.
- Carlisle, S. M., & Trevors, J. T. (1988). Glyphosate in the environment. *Water Air Soil Pollution*, 39, 409-420.
- Casler, M. D., & Jung, H. G. (2006). Relationships of fibre, lignin, and phenolics to in vitro fibre digestibility in three perennial grasses. *Animal Feed Science and Technology*, 125, 151-161.
- Cline, M. G. (1994). The Role of hormones in apical dominance. New approaches to an old problem in plant development. *Physiologia Plantarum*, 90, 230-237.
- Cooke, S. S. (1997). A field guide to the common wetland plants of Western Washington and Northwestern Oregon. *Seattle Audubon Society and Washington Native Plant Society*, (p. 417). Seattle.
- Costanza, R., d'Arge, R., de Groot, R., Farberk, S., Grasso, M., Hannon, B., . . . van den Belt, M. (1997). The value of the world's ecosystem services and



- natural capital. *Nature*, 387, 253-260. Retrieved from [http://www.esd.ornl.gov/benefits\\_conference/nature\\_paper.pdf](http://www.esd.ornl.gov/benefits_conference/nature_paper.pdf)
- Dow AgroScience LLC. (2009, May 29). Specimen Label: Rodeo. Indianapolis, Indiana, USA. Retrieved October 12, 2012, from Dow AgroScience LLC: [http://www.midwestaquacare.com/product-labels/rodeo\\_label.pdf](http://www.midwestaquacare.com/product-labels/rodeo_label.pdf).
- Eschenburg, S., Healy, M. T., & Priestman, M. A. (2002). How the mutation glycine96 to alanine confers glyphosate insensitivity to 5-enolpyruvyl shikimate-3-phosphate synthase from *Escherichia coli*. *Planta*, 216, 129-135.
- Federal Emergency Management Agency (FEMA). (2012, December 24). Google Earth kmz file for 100 year flood zone. Google.
- Fuchs, M. A., Geiger, D. R., Reynolds, T. R., & Bourque, J. E. (2002). Mechanisms of glyphosate toxicity in velvetleaf (*Abutilon theophrasti* medikus). *Pesticide Biochemistry and Physiology*, 74, 27-39.
- Galatowitsch, S. M., Anderson, N. O., & Ascher, P. D. (1999). Invasiveness in wetland plants in temperate North America. *Wetlands*, 19, 733-755.
- Galkin, S., Ämmälahti, E., Kilpeläinen, I., Brunow, G., & Hatakka, A. (1997). Characterisation of Milled Wood Lignin from Reed Canary Grass (*Phalaris arundinacea*). *Holzforschung - International Journal of the Biology, Chemistry, Physics and Technology of Wood*, 51, 130-134.
- Geiger, D. R., & Bestman, H. D. (1990). Self-limitation of herbicide mobility by phytotoxic action. *Weed Science*, 38, 324-329.
- Google Earth. (2012, December 12). *Google Earth*. Retrieved December 12, 2012, from Program for mapping sites and flood zones: <http://earth.google.com>
- Hall, S. J., & Zedler, J. B. (2010). Constraints on sedge meadow self-restoration in urban wetlands. *Restoration Ecology*, 18, 671-680.
- Healy, M. T., & Zedler, J. B. (2010). Setbacks in replacing *Phalaris arundinacea* monotypes with sedge meadow vegetation. *Restoration Ecology*, 18, 155-164.
- Hellqvist, S., Finell, M., & Landstrom, S. (2003). Reed canary grass--observations of effects on crop stand and fibre quality caused by infestation of *Epicalamus phalaridis*. *Agricultural Food Science*, 12, 49-56.

- Hoskins, B. (2002, January 21). *Organic matter by loss on ignition*. Retrieved August 12, 2012, from <http://www.naptprogram.org/files/napt/publications/method-papers/2002-organic-matter-by-loss-on-ignition.pdf>
- Howard, J. (2012). (D. L. Fong, Interviewer)
- Iannone, B. V., & Galatowitsch, S. M. (2008). Altering light and soil N to limit *Phalaris arundinacea* reinvasion in sedge meadow restorations. *Restoration Ecology*, 16, 689-701.
- Invasive Species Specialist Group. (2010). *ISSG*. Retrieved July 28, 2012, from Global Invasive Species Database: <http://www.issg.org/database/species/ecology.asp?si=394&fr=1&sts=&lang=EN>
- Johnson, C. A., & Zedler, J. B. (2012). Identifying preferential associates to initiate restoration plantings. *Restoration Ecology*, 20, 764-772.
- Kercher, S. M., & Zedler, J. B. (2004). Multiple disturbances accelerate invasion of reed canary grass (*Phalaris arundinacea* L.) in a mesocosm study. *Oecologia*, 138, 455-464.
- Kercher, S. M., Herr-Turoff, A., & Zedler, J. B. (2007). Understanding invasion as a process: the case of *Phalaris arundinacea* in wet prairies. *Biological Invasions*, 9, 657-665.
- Kim, K. D., Ewing, K., & Giblin, D. E. (2006). Controlling *Phalaris arundinacea* (reed canarygrass) with live willow stakes: A density-dependent response. *Ecological Engineering*, 27, 219-227.
- Klimešová, J. (1995). Population dynamics of *Phalaris arundinacea* L. and *Urtica dioica* L. in a floodplain during a dry period. *Wetlands Ecology and Management*, 3, 79-85.
- Lavergne, S., & Molofsky, J. (2004). Reed canary grass (*Phalaris arundinacea*) as a biological model in the study of plant invasions. *Critical Reviews in Plant Sciences*, 23, 415-429.
- Lyfod, M. (1993). Reed canary grass controls tested (Minnesota). *Restoration and Management Notes*, 11, 169.
- Mack, M. C., & D'Antonio, C. M. (1998). Impacts of biological invasions on disturbance regimes. *Trend Ecol. Evol.*, 13, 195-198.

- Marquis, L. Y., Comes, R. D., & Yang, C. P. (1979). Selectivity of glyphosate in creeping red fescue and reed canarygrass. *Weed Research*, 19, 335-342.
- Maslova, S. P., Tabalenkova, G. N., Kurenkova, S. V., & Plusnia, S. N. (2007). Seasonal changes in anatomical and morphological structure and the content of phytohormones and sugars in underground shoots of a long-rhizome perennial grass *Phalaroides arundinacea*. *Russian Journal of Plant Physiology*, 54, 491-497.
- Matthews, J. W., & Spyreas, G. (2010). Convergence and divergence in plant community trajectories as a framework for monitoring wetland restoration progress. *Journal of Applied Ecology*, 47, 1128-1136.
- Mauer, D. A., & Zedler, J. B. (2002). Differential invasion of a wetland grass explained by tests of nutrients and light availability on establishment and clonal growth. *Oecologia*, 131, 279-288.
- McIntyre, G. I. (1971). Apical dominance in the rhizome of *Agropyron repens*: Some factors affecting the degree of dominance in isolated rhizomes. *Canadian Journal of Botany*, 49, 99-109.
- Merigliano, M. F., & Lesica, P. (1998). The native status of reed canary grass (*Phalaris arundinacea* L.) in the inland Northwest, USA. *Natural Areas Journal*, 18, 223-230.
- Miller, T. W., Martin, L. P., & MacConnell, C. B. (2008). Managing reed canarygrass (*Phalaris arundinacea*) to aid in revegetation of riparian buffers. *Weed Technology*, 22, 507-513.
- Mitsch, W. J., & Gosselink, J. G. (2007). *Wetlands 4th Edition*. Hoboken: Wiley.
- Monsanto. (2010). Round-Up Pro Herbicide Label. St. Louis, MO, USA. Retrieved October 12, 2012, from <http://www.cdms.net/LDat/ld07A016.pdf>
- Nelson, N. F., & Dietz, R. H. (1966). Cattail control methods in Utah. *Utah State Department of Fish and Game*, (p. Publication 66). Salt Lake City.
- NuFarm Americas Inc. (2008). Aqua-Neat Herbicide Label. Burr Ridge, IL, USA. Retrieved October 12, 2012, from <http://www.cdms.net/LDat/ld5NE005.pdf>
- NuFarm Americas Inc. (2009). Razor Pro Herbicide Label. Burr Ridge, IL, USA. Retrieved October 12, 2012, from <http://www.cdms.net/LDat/ld6CS015.pdf>

- Ohio Department of Natural Resources. (2000). *ODNR*. Retrieved May 28, 2012, from Ohio's Invasive Plant Species:  
[http://ohiodnr.com/Portals/3/invasive/pdf/OHIO%20INVASIVE%20PLANT S.pdf](http://ohiodnr.com/Portals/3/invasive/pdf/OHIO%20INVASIVE%20PLANT%20S.pdf)
- Osborn, L. (n.d.). *Current Results Nexus*. Retrieved 18 October, 2012, from Current Results:  
<http://www.currentresults.com/Weather/Ohio/temperature-april.php>
- Page, R. A., Okada, S., & Harwood, J. L. (1994). Acetyl-CoA carboxylase exerts strong flux control over lipid synthesis in plants. *Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism*, 1210, 369-372.
- Perkins, T. E., & Wilson, M. V. (2005). The impacts of *Phalaris arundinacea* (reed canarygrass) invasion on wetland plant richness in the Oregon Coast Range, USA depend on beavers. *Biological Conservation*, 124, 291-295.
- Perry, L. G., & Galatowitsch, S. M. (2006). Light competition for invasive species control: A model of cover crop- weed competition and implications for *Phalaris arundinacea* control in sedge meadow wetlands. *Euphytica*, 148, 121-134.
- Perry, L. G., Galatowitsch, S. M., & Rosen, C. J. (2004). Competitive control of invasive vegetation: a native wetland sedge suppresses *Phalaris arundinacea* in carbon-enriched soil. *Journal of Applied Ecology*, 41, 151-162.
- Pimental, D., McNair, S., Janecka, J., Wightman, J., Simmonds, C., O'Connell, C., . . . Tsomondo, T. (2001). Economic and environmental threats of alien plant, animal, and microbe invasions. *Agriculture, Ecosystems and Environment*, 84, 1-20.
- Prasser, N., & Zedler, J. B. (2010). Salt tolerance of invasive *Phalaris arundinacea* exceeds that of native *Carex stricta* (Wisconsin). *Ecological Restoration*, 28, 238-240.
- Rubin, J. L., Gaines, C. G., & Jensen, R. A. (1984). Glyphosate inhibition of 5-enolpyruvylshikimate 3-phosphate synthase from suspension-cultured cells of *Nicotiana glauca*. *Plant Physiology*, 75, 839-845.
- Sahramaa, M., & Jauhiainen, L. (2003). Characterization of development and stem elongation of reed canary grass under northern conditions. *Industrial Crops and Products*, 18, 155-169.

- Sale, P. J., & Wetzel, R. G. (1983). Growth and metabolism of *Typha* species in relation to cutting treatments. *Aquatic Botany*, 15, 321-334.
- Shaner, D. (2006). An overview of glyphosate mode of action: why is it such a great herbicide? *North Central Weed Science Society Proceedings*, 61, p. 94. Ft. Collins. Retrieved August 5, 2012, from <http://www.ncwss.org/proceed/2006/abstracts/94.pdf>
- Sheaffer, C. C., Rosen, C. J., & Gupta, S. C. (2008). Reed canarygrass forage yeiled and nutrient uptake on a year-round wastewater application site. *Journal of Agronomy and Crop Science*, 194, 465-469.
- Sprankle, P., Penner, D., & Meggitt, W. F. (1975). Absorption, action, and translocation of glyphosate. *Weed Science*, 23, 235-238.
- Spuhler, D. R. (1994). Low plant diversity found in communities dominated by reed canary grass (*Phalaris arundinacea*). *Proceedings of the 14th annual North American Prairie Conference*, (pp. 43-47).
- Steinrücken, H. C., & Amrhein, N. (1980). The herbicide glyphosate is a potent inhibitor of 5-enolpyruvyl-shikimic acid-3-phosphate synthase. *Biochemical and Biophysical Research Communications*, 94, 1207-1212.
- Stevens, J. M., & Fehmi, J. S. (2011). Early establishment of a native grass reduces the competitive effect of a non-native grass. *Restoration Ecology*, 19, 399-406.
- Stoller, Inc. (2005). X-Cyte Label. Houston, TX, USA. Retrieved October 12, 2012, from <http://stollerusa.com/sites/default/files/pdf/labelinfo13551.pdf>
- Syngenta. (2009). Fusillade II Label. Greensboro, NC, USA. Retrieved October 12, 2012, from <http://www.gemplers.com/docs/IS/g49785.pdf>
- Tencoz, Inc. (2008). Buccaneer 5 Label. Alpharetta, GA, USA. Retrieved October 12, 2012, from <http://www.cdms.net/LDat/ld83M005.pdf>
- Tu, M., Hurd, C., & Randall, J. M. (2001). *The Nature Conservancy*. Retrieved May 24, 2012, from Weed Control Methods Handbook. Toolds and techniques for use in natural areas.: <http://www.invasive.org/gist/handbook.html>
- United States Department of Agriculture. (2012). *USDA*. Retrieved July 28, 2012, from Plants profile for *Phalaris arundinacea*: <http://plants.usda.gov/java/profile?symbol=PHAR3>

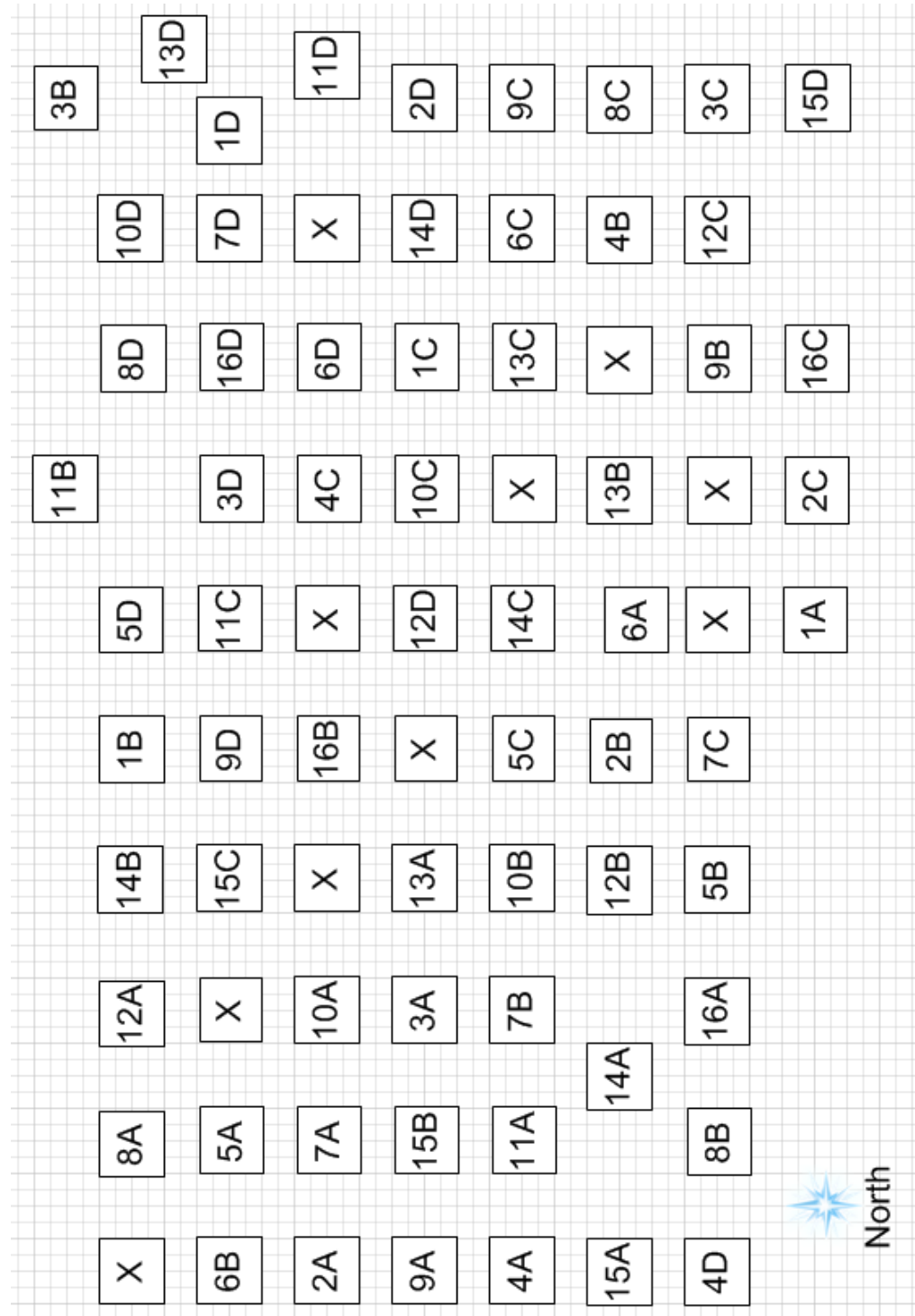
- US Army Corps of Engineers. (1995). *Wetland Delineation Manual*. Retrieved July 28, 2012, from <http://www.wetlands.com/coe/87manapc.htm>.
- Vitousek, P. M., D'Antonio, C. M., Loope, L. L., & Westbrooks, R. (1996). Biological invasions as global environmental change. *Conservation and Ecosystem Management*, 218-228.
- Vymazal, J., Kröpfelová, L., Švehla, J., & Štíć, J. (2010). Can multiple harvest of aboveground biomass enhance removal of trace elements in constructed wetlands receiving municipal sewage? *Ecological Engineering*, 36, 939-945.
- Walter, K. A., Ridley, S. M., & Harood, J. L. (1988). Effects of the selective herbicide fluazifop on fatty acid synthesis in pea (*Pisum sativum*) and barley (*Hordeum vulgare*). *Biochem Journal*, 254, 811-817.
- Wetzel, P. R., & van der Valk, A. G. (1998). Effects of nutrient and soil moisture on competition between *Carex stricta*, *Phalaris arundinacea*, and *Typha latifolia*. *Plant Ecology*, 138, 179-190.
- Wisconsin Reed Canary Grass Management Working Group. (2009). *Reed Canary Grass (Phalaris arundinacea) Management Guide: Recommendations for Landowners and Restoration Professionals*. Retrieved December 12, 2012, from [ftp://ftp-fc.sc.egov.usda.gov/WA/Tech/RCG\\_management\\_0509.pdf](ftp://ftp-fc.sc.egov.usda.gov/WA/Tech/RCG_management_0509.pdf)

## Appendices

### Appendix 1. Timeline of treatment plan.

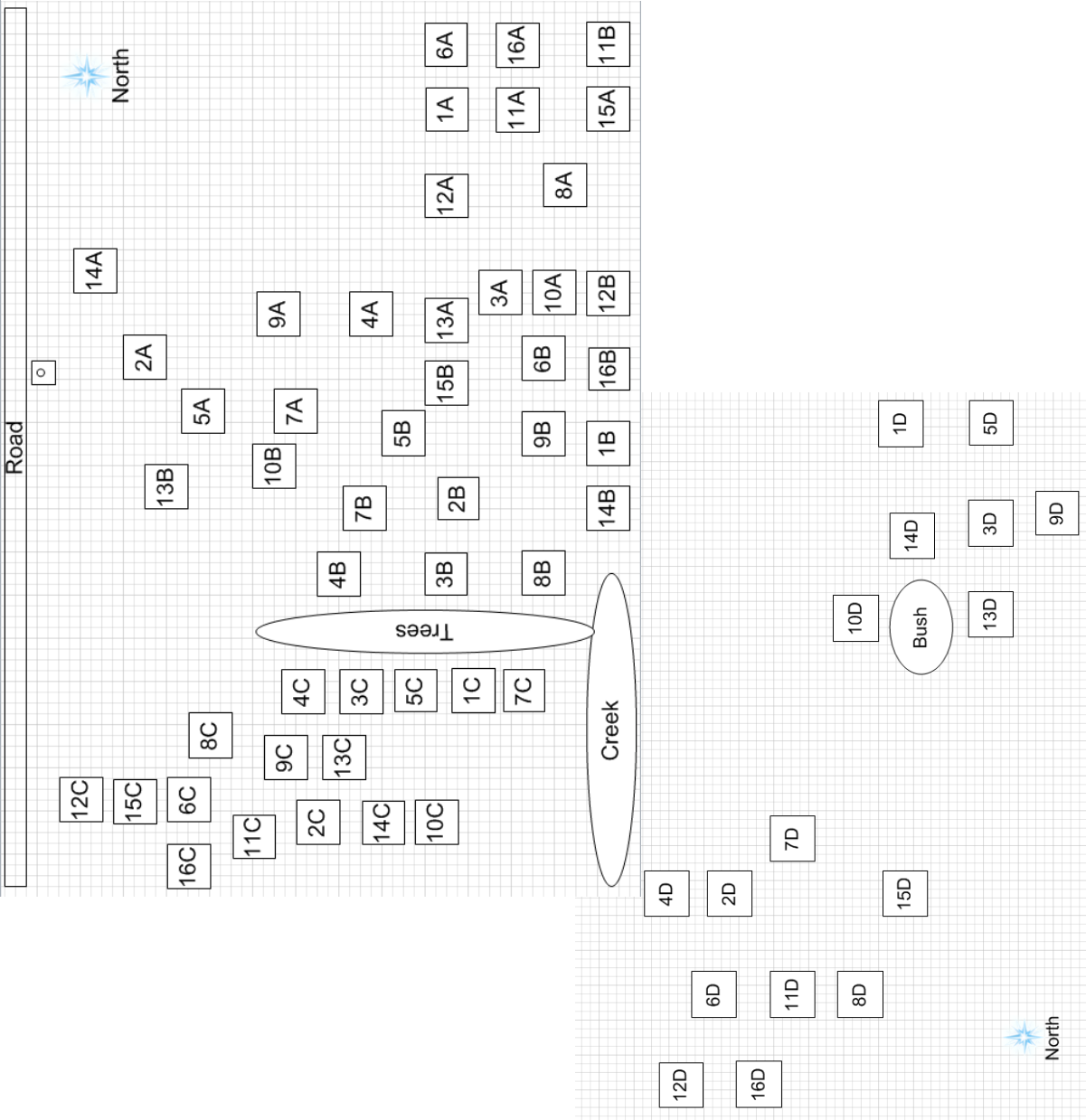
May 9, 2011	Kinetin App Flat Site- Spring Only and Spring & Fall
May 11, 2011 Fall	Kinetin App Sloped Site- Spring Only and Spring & Fall
May 28, 2011	Gly. App Flat Site- Spring Only and Spring & Fall
May 28, 2011	Gly. App Sloped Site- Spring Only and Spring & Fall
May 28, 2011	Fluaz. App Flat Site- Spring Only and Spring & Fall
May 28, 2011 Fall	Fluaz. App Sloped Site- Spring Only and Spring & Fall
June 24, 2011	Percent cover taken of Spring Only and Spring & Fall
July 13, 2011	Percent cover taken of Spring Only and Spring & Fall
August 23, 2011	Percent cover taken of Spring Only and Spring & Fall
August 26 & Sept 11, 2011	Kinetin App Flat Site- Fall Only and Spring & Fall
August 26 & Sept 11, 2011	Kinetin App Sloped Site- Fall Only and Spring & Fall
October 11, 2011	Gly. App Flat Site- Fall Only and Spring & Fall
October 11, 2011	Gly. App Sloped Site- Fall Only and Spring & Fall
October 11, 2011	Fluazifop App Flat Site- Fall Only and Spring & Fall
October 11, 2011	Fluaz. App Sloped Site- Fall Only and Spring & Fall
October 21, 2011	Percent cover taken of Fall Only and Spring & Fall
November 9, 2011	Percent cover taken of Fall Only and Spring & Fall
December 8, 2011	Harvest of Spring Only Treatments
February 22, 2012	Kinetin sprayed in greenhouse
March 7, 2012	Fluazifop-p-butyl sprayed in greenhouse
April 10, 2012	Started stratifying seeds
April 12, 2012	% cover of Fall Only and Spring & Fall Treatment
April 13, 2012	Harvest of Fall Only and Spring & Fall Treatments
May 10, 2012	Planted seed mix

## Appendix 2. Plot arrangement of flat site.





Appendix 3. Plot arrangement of sloped site.



#### Appendix 4. Calculation of Spring Biomass.

1. Lay out harvested biomass on newspapers
2. Separate RCG matter from non-RCG matter
3. Determine “air weight” of RCG when grass is dry to touch
4. Mill RCG to homogenize sample.
5. Record “total milled weight.”
6. Find weight of aluminum dish “dish weight.”
7. Fill dish with 3.0 g milled grass.
8. Place in drying oven at 80°C for 48 hours until sample reaches a constant weight.
9. Weigh sample and record “dry weight + dish.”
10. Subtract “dish weight” from “dry weight + dish” to get “dry weight.”
11. Repeat steps 7-10 with 3 more replicates.
12. Calculate the average dry weight and determine standard deviation.
13. When standard deviation showed to be <0.01 g for 12 samples, there was no need to perform 4 replicates of measurement.
14. Fill dish with at least 4.0 g of milled grass to determine “milled grass weight.”
15. Perform steps 8-10 with remaining plot samples.
16. Place dried samples into a muffle furnace at 480°C for 4 hours.
17. Record “ash weight + dish weight.”
18. Subtract “dish weight” from “ash weight + dish weight” to get “ash weight.”
19. Subtract “ash weight” from “dry weight” to get “organic weight.”
20. Divide “organic weight” by “dry weight” to find portion of organic matter in dry weight.
21. Multiply portion of organic matter in dry weight by total milled weight to get amount of organic matter in a quadrat (1.5 m x 1.5 m or 5 ft. x 5 ft.).

Calculation of Fall Biomass utilized the same procedure with the exception of drying all RCG matter to a constant weight in step 8 and then proceeding to step 4 to milling the entire dried sample.

**Appendix 5. Initial plant assessment of flat site and sloped site on May 1, 2011 before treatment regimen by summation of each quadrat regardless of treatment. Note: Percentage may not add up to 100% because each species in the quadrat was estimated to the nearest 5%.**

Plant name		Flat site	Sloped site
Reed Canary Grass	<i>Phalaris arundinacea</i> L.	97.36%	90.80%
Wingstem Sunflower	<i>Verbesina alternifolia</i> L.	0%	19.87%
Stinging Nettle	<i>Urtica dioica</i> L.	0%	5.34%
Canada Thistle	<i>Cirsium arvense</i> L.	1.25%	0%
Green Headed Coneflowers	<i>Rudbeckia laciniata</i> L.	1.02%	0%
Common Ragweed	<i>Ambrosia artemisiifolia</i> L.	0.57%	0%
Dogbane	<i>Apocynum cannabinum</i> L.	0.34%	0%
Giant Ironweed	<i>Vernonia gigantea</i> L.	0%	0%
Field Bindweed	<i>Convolvulus arvensis</i> L.	0%	0%

Species Richness	5	3
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**Appendix 6. Percent coverage assessment of flat site compared to sloped site on August 10, 2011 by summation of each quadrat regardless of treatment.**

Plant Name		Flat site	Sloped site
Reed Canary Grass	<i>Phalaris arundinacea</i> L.	47.61%	54.34%
Canada Thistle	<i>Cirsium arvense</i> L.	6.39%	0%
Stinging Nettle	<i>Urtica dioica</i> L.	6.25%	1.14%
Wingstem Sunflower	<i>Verbesina alternifolia</i> L.	1.82%	12.23%
Giant Ironweed	<i>Vernonia gigantea</i> L.	1.22%	0.68%
Common Ragweed	<i>Ambrosia artemisiifolia</i> L.	1.02%	0%
Green Headed Coneflowers	<i>Rudbeckia laciniata</i> L.	1.02%	0%
Dogbane	<i>Apocynum cannabinum</i> L.	0.02%	0%
Poison Hemlock	<i>Conium maculatum</i> L.	0%	4.14%
Field Bindweed	<i>Convolvulus arvensis</i> L.	0%	1.02%

Richness	8	6
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Appendix 7. Percent cover results of a four-way factorial ANOVA shows that herbicide type had the greatest influence on RCG percent cover regrowth. The only interaction between the four factors tested was between site type and herbicide type.

Source	Type III SS	Mean Square	F Value	Pr > F
Site	451.5625	451.5625	0.77	0.3817
Kinetin	416.8403	416.8403	0.71	0.4006
Herb	136129.1667	68064.5833	116.11	<.0001
Time	2.7917	1.3958	0.00	0.9976
site*herb	11722.6667	5861.3333	10.00	<.0001

Appendix 8. Field observations one year after last biomass harvest recorded by Amon (2010-2012).

Date of observations: 4/18/2013

Site	Water level	RCG stage	RCG height	Management notes
Rotary Park	no flood	3 to 4 leaf	30 cm	
Phillips Park sloped site	no flood	4 to 5 leaf	35 cm	some signs of continued suppression
Phillips Park flat site	4cm flood	3 to 4 leaf	40 cm	minimal signs of continued suppression
Phillips Park	deep water (30-35 cm deep)	5 leaf		RCG have thicker, fatter stems compared to other sites
McIntire Property	fully saturated soil	3 to 5 leaf	20-30 cm	variable growth
Siebenthaler Fen	some flood (5-8 cm deep)	5 to 7 leaf	35-45 cm	

Notes in support from Amon lab

On April 18, 2013, several RCG sites in the approximately 2000 acres (hectare 809.371) Beaver Creek Wetland complex (studied in this thesis) were examined to determine their readiness for herbicide treatment. RCG at these sites began noticeably growing on about April 1, 2013. Both on saturated and unsaturated sites were examined. Two adjacent sites that were well drained and not saturated or above the surface had RCG in the 3 to 4 and 4 to 5 leaf stages, with varying heights from 30-35 cm tall.

On a flat site within 200 to 400 meters away, water was 4-6 cm deep and RCG had 3 to 4 leaves with an average height of 40 cm. In the same area a deeper water (30-35 cm) had comparatively robust stems at the 5 leaf stage that were emergent and about 50 cm tall. A site 2 km north of that site, a groundwater seepage site had plants at the 3 to 5 leaf stage that were only 20-30 cm tall. Another 2 km north, a flat site flooded (5-8 cm) with rainfall was at the 5 to 7 leaf stage and ranged from 35-45 cm tall.

As one can see sites located close to one another can be subject to quite different water regimens and plant characteristics. A manager attempting to treat these sites with herbicide has a number of confounding situations to solve. Application of kinetin suggested by Annen (2010) would present serious timing problems. The grass grows at a rate that would not leave time for both kinetin and herbicide treatment before it gets beyond his ideal 3 to 4 leaf stage, and several neighboring populations are in different stages of readiness on the same day. Also some plants are flooded, preventing use of sethoxydim or fluazifop

and probably lessening the contact of herbicide on leaves if glyphosate was used. Rainfall interval of 5 to 10 days during this time of year further prevent application of herbicide.

The result of these problems is a need to apply in non-optimal stages of RCG. My study had to wait until plants were nearly a meter tall (some more) before conditions permitted kinetin or herbicide application. Those applications apparently did not allow kinetin to enhance the herbicide effectiveness. The herbicide did however produce a easily visible top kill of the plants and the above ground stems appeared to dry without producing shoots from their nodes. I must assume that the less than total kill measured by biomass was the result of unaffected rhizomes.

## Appendix 9: Full statistical report performed by Bev Grunden of the Wright State Statistical Counseling Center.

### DATA ANALYSIS:

Data were collected in plots of ground containing Reed Canary Grass. Two outcomes were measured on these plots: percent coverage and biomass. The independent variables (factors) were:

- **Herbicide** (3 levels: glyphosate, fluazifop, and control)
- **Site** type (2 levels: flat and slope)
- Use of **Kinetin** (2 levels: yes and no)
- **Time** of treatment (3 levels: Spring, Fall, and Both)

There were 4 replicates for each factor combination of the four variables listed above.

Your research question sought to determine which treatment combination would provide the best method of controlling the Reed Canary Grass. An overall level of significance of  $\alpha_{\text{overall}} = 0.05$  was used for all tests of hypothesis, with a test-wise level of significance =  $\alpha_{\text{test}} = 0.05/3 = 0.0167$ .

### PERCENT COVERAGE

A four-way factorial ANOVA was run to analyze this outcome measure. The model p-value was  $p < 0.0001$ . Only one of the interactions was significant, **site\*herbicide**. The only main effect that showed significance was **herbicide**. I removed all insignificant interactions and re-ran the model with only the main effects and the interaction site\*herbicide. The results are shown below.

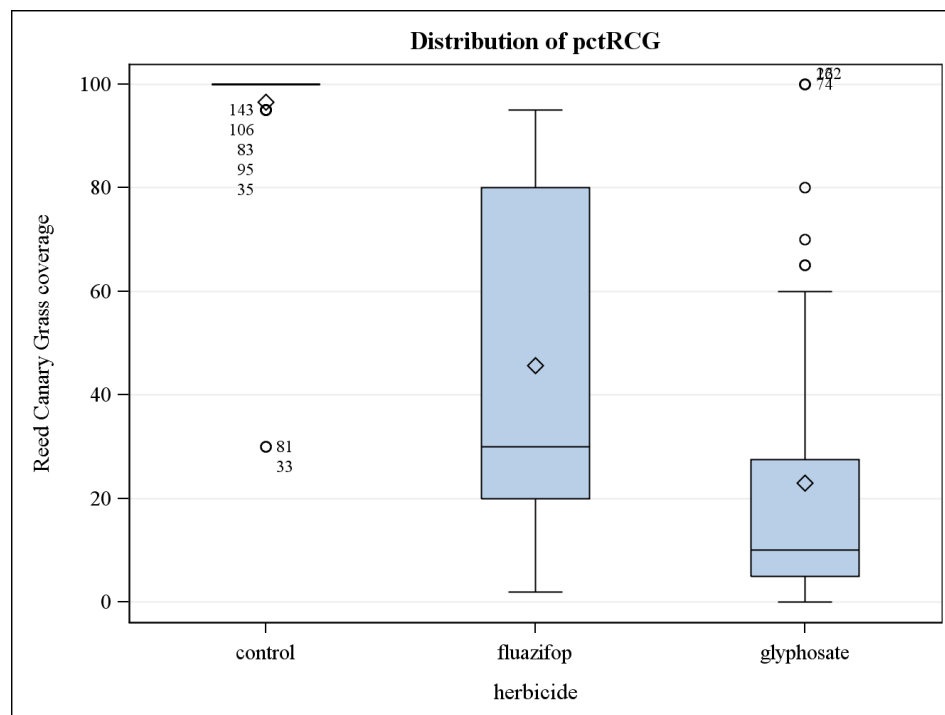
Source	DF	Type III SS	Mean Square	F Value	Pr > F
site	1	451.5625	451.5625	0.77	0.3817
kinetin	1	416.8403	416.8403	0.71	0.4006
herb	2	136129.1667	68064.5833	116.11	<.0001
time	2	2.7917	1.3958	0.00	0.9976
site*herb	2	11722.6667	5861.3333	10.00	<.0001

The significant interaction between site type and herbicide level suggests that the differences among herbicide levels may not be the same for both flat and sloped terrains. However, the main effect for herbicide is quite strong and suggests that, regardless of site type, there is some sort of significant difference among the herbicide levels.

Let's begin by analyzing the main effect. If we ignore the site type, we see that not all the herbicide levels have the same mean percent coverage ( $p < 0.0001$ ). Post hoc tests will tell us how they differ.

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	herb
A	96.563	48	control
B	45.729	48	fluazifop
C	23.021	48	glyphosate

The Tukey HSD test (see table above) indicates that all three means differ from each other significantly (because each has its own Tukey grouping letter. We see that the glyphosate was more effective in reducing the RCG than both the fluazifop and control, and the fluazifop was more effective than control. Side-by-side box plots shown below illustrate the differences we found in the Tukey post hoc test.



Now let's consider the significant interaction ( $p < 0.0001$ ). It would seem that the differences in mean by site are not the same for all three herbicide levels. The means by treatment combination are shown below.



Analysis Variable : pctRCG Reed Canary Grass coverage				
herbicide	site type	N Obs	Mean	Std Dev
control	flat	24	99.79	1.021
	slope	24	93.33	19.597
glyphosate	flat	24	8.58	5.356
	slope	24	37.46	35.268
fluazifop	flat	24	51.63	34.020
	slope	24	39.83	25.380

Pairwise comparisons of these six means were performed, while adjusting for multiple testing using the Tukey method. The means are shown below in ascending order:

Treatment Combination	Mean	Std Dev
Control/Flat	99.79	1.021
Control/Slope	93.33	19.597
Fluazifop/Flat	51.63	34.020
Fluazifop/Slope	39.83	25.380
Glyphosate/Slope	37.46	35.268
Glyphosate/Flat	8.58	5.356

In the table above, means that were not found to be significantly different were shaded by the same color. Any two means that are of different colors were found to be significantly different. For example, the mean percent coverage after the Glyphosate herbicide treatment on the flat terrain was found to be significantly less than that for Glyphosate herbicide treatment on the sloped terrain.

No other main effects were significant (site type, use of kinetin, and time of treatment).

## SPRING BIOMASS

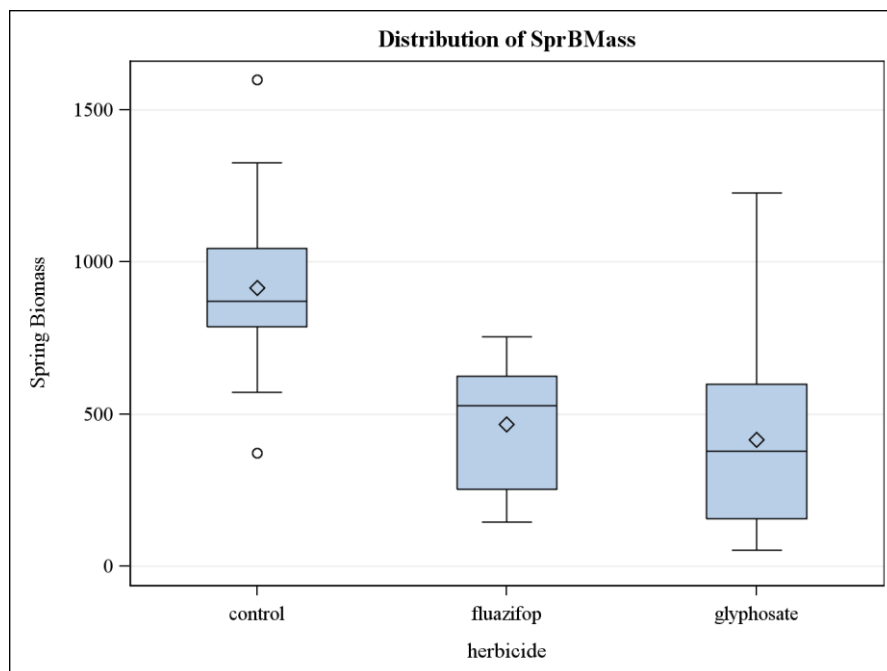
The biomass for the Spring plots was measured. A three-way factorial ANOVA was run to determine which treatment combination was most effective at controlling the RCG, as measured by biomass. The model p-value was  $p < 0.0001$ . For this outcome, none of the interactions were significant. I re-ran the model using only the main effects. The results are shown below:

Source	DF	Type III SS	Mean Square	F Value	Pr > F
site	1	54203.954	54203.954	0.78	0.3822
kinetin	1	169080.897	169080.897	2.43	0.1262
herb	2	2415140.596	1207570.298	17.37	<.0001

From these results we conclude that only the main effect for herbicide is significant ( $p < 0.0001$ ).

It does not appear that the site type and the use of kinetin had an effect on the mean biomass, as measured in the Spring.

A side-by-side box plot (see below) shows how a comparison of the distributions of the biomass measures for the three herbicide levels. Visually, it appears that both herbicides produced lower biomass means than the control. But post hoc tests, using the Tukey method, were run to verify which means were different.



Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	herb
A	914.76	16	control
B	465.82	16	fluazifop
B	415.95	16	glyphosate

The Tukey grouping table above shows that the mean biomass measures for both herbicides were significantly lower than the mean for the control, but we cannot conclude that the mean biomass differs between the two herbicides.

### FALL BIOMASS

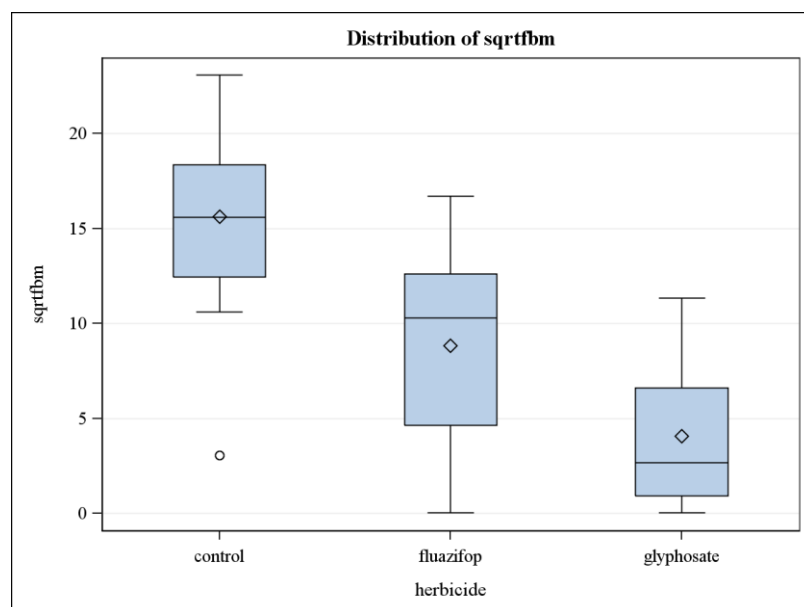
The biomass for the Fall treatment and Spring+Fall treatment plots was measured. Because we had two different treatment times in this model, time was kept in the model. A four-way factorial ANOVA was run to determine which treatment combination was most effective at controlling the RCG, as measured by biomass. The model p-value was  $p < 0.0001$ . For this outcome, none of the interactions were significant. I re-ran the model using only the main effects. The results are shown below:

Source	DF	Type III SS	Mean Square	F Value	Pr > F
site	1	1.541132	1.541132	0.08	0.7792
kinetin	1	0.701644	0.701644	0.04	0.8499
herb	2	2149.862585	1074.931292	55.17	<.0001
time	1	17.164595	17.164595	0.88	0.3505

From these results we conclude that only the main effect for herbicide is significant ( $p < 0.0001$ ).

It does not appear that the site type, use of kinetin, or number of treatments (fall vs spring + fall) had an effect on the mean biomass.

A side-by-side box plot (see below) shows a comparison of the distributions of the biomass measures for the three herbicide levels. Visually, the three means appear to be different, but post hoc tests, using the Tukey method, were run to verify which means were different.



Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	herb
A	15.614	32	control
B	8.844	32	fluazifop
C	4.081	32	glyphosate

We conclude that the mean biomass, as measured in the fall, is significantly greater for the control group than either of the herbicides. And the mean biomass for fluazifop is significantly greater than for glyphosate.

### ADDITIONAL DATA ANALYSES:

Means and standard deviations were calculated on to obtain the mean biomass for spring and fall control plots. The mean biomass for spring control was 914.758 and for fall control was 262.510.

herbicide	N Obs	Variable	Label	Mean	Std Dev
control	48	SprBMass	Spring Biomass	914.758	288.283
		FBMass	Fall Biomass	262.510	131.324

If we normalize the biomass measures for the herbicide groups by dividing by the appropriate mean for control, we have a new variable that represents the percent

coverage remaining (PCR). For this variable it is also true that a smaller number represents better control of RCG, however, it has been adjusted for the amount of RCG present in an uncontrolled setting, as measured by the control plots.

$$\text{For spring biomass measurements, PCR} = 100 \left( \frac{\text{spring biomass}}{914.758} \right)$$

$$\text{For fall biomass measurements, PCR} = 100 \left( \frac{\text{fall biomass}}{262.510} \right)$$

Descriptive statistics were generated for PCR measurements by herbicide, site type, and treatment time:

Analysis Variable : PCR								
herbicide	site type	time	N Obs	Median	Mean	Std Dev	Minimum	Maximum
glyphosate	flat	both	8	0.144	0.864	1.376	0.000	3.966
		spring	8	31.550	33.487	21.355	5.917	71.127
		fall	8	4.206	8.603	9.654	1.517	28.452
	slope	both	8	22.634	22.186	22.514	0.000	48.936
		spring	8	62.864	57.455	39.780	9.055	134.138
		fall	8	11.638	13.793	14.533	0.431	43.937
fluazifop	flat	both	8	44.662	45.267	40.915	0.000	98.105
		spring	8	63.946	55.603	21.467	23.802	81.290
		fall	8	47.263	48.012	12.232	30.516	61.422
	slope	both	8	7.361	18.805	23.161	1.312	62.422
		spring	8	49.996	46.243	21.897	15.778	82.495
		fall	8	38.551	42.960	35.267	0.000	106.263

To explore the effects of herbicide, site type, kinetin and treatment time, an ANOVA was run using these four variables as fixed factors and PCR as the dependent variable or outcome. Model assumptions were not satisfied until a log transformation was applied to the PCR variable. A level of significance  $\alpha = 0.05$  was used for all tests of hypothesis.

## **RESULTS:**

The model containing all four factor variables (herbicide, site type, kinetin, and time) was significant ( $p < 0.0001$ ). Interaction effects that were not considered important were removed from the model. Main effects and interactions with  $p$ -values  $< 0.15$  were retained in the model. The reduced model produced these  $p$ -values. Those that are significant at the 0.05 level are highlighted.

- Site ( $p = 0.4577$ )
- Kinetin ( $p = 0.7435$ )
- Herbicide ( $p < 0.0001$ )
- Time ( $p < 0.0001$ )
- Site\*Herbicide ( $p = 0.0072$ )
- Herbicide\*Time ( $p = 0.0759$ )

**Site\*Herbicide** – this interaction effect was significant, indicating that the effect of site type may not be the same for both herbicides.

- By site type:
  - Flat sites: Means between herbicides are significantly different ( $p < 0.0001$ )
  - Slope sites: Means between herbicides are not significantly different ( $p = 0.2262$ )
  - See means in the table below:

Analysis Variable : PCR						
site type	herbicide	N Obs	Mean	Std Dev	Minimum	Maximum
flat	glyphosate	24	14.318	19.232	0.000	71.127
	fluazifop	24	49.627	26.744	0.000	98.105
slope	glyphosate	24	31.144	32.765	0.000	134.138
	fluazifop	24	36.003	29.050	0.000	106.263

- We can see that the means between the two herbicides are significantly different for the flat sites, but not for the slope sites, hence the significant interaction. The main effect for herbicide is strongly influenced by the results on the flat plots, and less so for the slope plots. We will not analyze the main effect for herbicide.
- By herbicide:

- Glyphosate: Means between site types are significantly different ( $p = 0.0153$ )
- Fluazifop: Means between site types are not significantly different ( $p = 0.1597$ )
- See means in the table below (notice that they are the same means in the table above, but we are comparing them differently):

Analysis Variable : PCR						
herbicide	site type	N Obs	Mean	Std Dev	Minimum	Maximum
glyphosate	flat	24	14.318	19.232	0.000	71.127
	slope	24	31.144	32.765	0.000	134.138
fluazifop	flat	24	49.627	26.744	0.000	98.105
	slope	24	36.003	29.050	0.000	106.263

- We can see that the means between the two site types are significantly different for glyphosate, but not for fluazifop, hence the significant interaction. The main effect for herbicide is strongly influenced by the results on the plots treated with glyphosate, but less so for the plots treated with fluazifop.

**Time** – this main effect is significant ( $p < 0.0001$ ), but is not included in any significant interaction terms, so we will explore how the means differ by time as a main effect.

- Treating the plots twice (both spring and fall) generated the smallest mean PCR: 21.781
- Treating the plots in the fall generated the next best result: mean PCR = 28.342
- Treating the plots in the spring generated the largest results: mean PCR = 48.197.
- All three means were significantly different from each other
  - Mean PCR for both treatments  $<<$  mean PCR for fall treatments ( $p = 0.0129$ )
  - Mean PCR for both treatments  $<<$  mean PCR for spring treatments ( $p < 0.0001$ )
  - Mean PCR for fall treatments  $<<$  mean PCR for spring treatments ( $p = 0.0052$ )

Analysis Variable : PCR						
time	N Obs	Median	Mean	Std Dev	Minimum	Maximum
both	32	4.308	21.781	29.523	0.000	98.105
spring	32	46.784	48.197	27.672	5.917	134.138
fall	32	22.330	28.342	26.335	0.000	106.263